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THE COLLAPSE PRODUCED BY VENOUS CONGESTION OF THE EXTREMITIES OR BY VENESECTION FOLLOWING CERTAIN HYPOTENSIVE AGENTS [1]

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It is well recognized that interruption of the lower thoracic and lumbar sympathetic nerves, or the administration of certain hypotensive drugs particularly sodium nitrite and the "sympatholytic" agents may cause hypotension and collapse in the erect position. It has been postulated that this hypotensive reaction is due to pooling of blood in the dependent parts of the body, particularly in the splanchnic vascular bed with consequent failure of venous return and, hence, of cardiac output (1).

During studies on the hemodynamic effects of hypotensive drugs in man we have observed sudden and marked reductions of arterial pressure frequently with collapse occurring in the supine position. This phenomenon was induced by subjecting the limbs to brief periods of venous congestion, after the administration of certain hypotensive agents (2). This paper presents data on the types of agents that cause this phenomenon as well as an analysis of the mechanisms whereby the effect is produced.

MATERIALS AND METHODS

The subjects were patients both hypertensive and normotensive from the hospital wards. Arterial pressure was recorded either by the usual auscultatory method or optically with a Hamilton manometer connected to an 18 gauge needle inserted into the brachial artery.

With the patient in the supine position, inflatable cuffs connected to a large air reservoir bottle were applied proximally to both thighs and one arm and were held in place by covering the cuffs and adjacent skin with gauze bandage. In all of the hypertensive patients the limbs were congested with a pressure of 100 mm. Hg and in the normotensive subjects at pressures of 60 to 80 mm. Hg. Before and after the administration of each of the various drugs tested the extremities were congested for five minutes unless collapse supervened. After each drug the blood pressure was recorded frequently. The point at which the blood pressure leveled off or began to rise was taken as the point for applying venous congestion. A similar procedure was used in the venesection experiments.

The volume of blood trapped in the congested extremities was determined according to the method of Ebert and Stead (3) following a congestion period of five minutes, instead of seven to 10 minutes as used by those investigators. The shorter congestion period may account for the fact that in the present experiments the volume of blood trapped in the extremities usually was smaller than that observed by Ebert and Stead.

Following the congesting period the pressure in the cuffs was raised to 250 mm. Hg after which the dye T-1824 was injected. Samples were drawn at eight and 10 minutes afterwards. The occluding cuffs were then released and further sampling was carried out at 18 and 20 minutes. The latter samples were used to determine total blood volume while the former were used to determine the volume of that portion of the body not removed from the general circulation by the occluding cuffs. It was found that the dye density values of the eight and 10 minute samples agreed closely with each other indicating that mixing was essentially complete at the end of eight minutes. Similarly the 18 minute were in good agreement with the 20 minute samples. The results of other investigations (4–7) also indicate that in normal subjects equilibration of dye is complete in less than eight minutes. Hence, it seems probable that this method was sufficiently accurate for the present purposes.

The segment plethysmograph used to determine changes in calf volume was the same as that described by Wilkins and Eichna (8) with the exception that proximal and distal blood pressure cuffs were not applied since in the present experiments the plethysmograph was used to determine volume changes rather than blood flow.

In the venesection experiments a 15 gauge needle was inserted into an antecubital vein and the blood introduced

[1] Supported in part by research grants from the National Heart Institute, U. S. Public Health Service, E. R. Squibb and Sons, New York, N. Y., and Irwin, Neisler and Company, Decatur, Ill.

[2] Captain, M.C., U.S.A.F.

into a vacuum flask containing 130 cc. of acid citrate dextrose solution (ACD). Immediately following completion of the bleeding period the flask was inverted, and then pressurized by raising it and introducing air into it in order to hasten the return of blood to the circulation.

RESULTS

Effect of various hypotensive agents in the production of collapse during venous congestion of the extremities

In the control period, prior to the administration of any drug only two of the 24 subjects exhibited a significant reduction of arterial pressure during congestion of the extremities for a five minute period. In these two cases (G. S. and J. L., Table I) the reductions in average $\left(\frac{\text{systolic} + \text{diastolic}}{2}\right)$ arterial pressure were 10 and 12 per cent, respectively. Neither of these patients exhibited signs of collapse.

Five patients who received sodium nitrite in doses of 90 to 210 mg. by mouth exhibited changes in average arterial pressure varying from + 1 to − 44 per cent (mean − 25 per cent) during congestion of the extremities. Three of these subjects developed marked hypotension and symptoms of collapse within two to three minutes after the congesting pressures were applied.

Five subjects were given 0.5 mg. of dihydroergocornine (DHO) intravenously. During congestion the changes in average arterial pressure in these patients varied between + 6 and − 48 per cent (mean − 27 per cent). Three of these subjects developed signs of collapse prior to the end of the five minute congesting period.

In three subjects given tetraethylammonium (250 to 400 mg. intravenously) none developed collapse during congestion of the limbs although all exhibited reductions in average arterial pressure varying from − 4 to − 22 per cent (mean − 15 per cent). However, all of the four subjects given the longer acting and more potent ganglionic blocking agent hexamethonium (C_6) developed collapse within two to five minutes of venous congestion. The reduction of arterial pressure in this group of patients varied between − 16 and − 56 per cent (mean − 37 per cent).

TABLE I

The effect of various hypotensive agents on the arterial pressure during congestion of the extremities of unsympathectomized patients

Pt.	Sex	Age	Drug	Dose and route	Control			Post-drug period			
					Arterial pressure		Change mean* arterial pressure	Arterial pressure		Change mean arterial pressure	Collapse
					Basal	After five min. of congestion		Basal	After five min. or less of congestion		
					mm. Hg	*mm. Hg*	*per cent*	*mm. Hg*	*mm. Hg*	*per cent*	
R. M.	M	24	Sodium nitrite	180 mg. P.O.	200/120	190/112	− 5	180/106	155/100	−11	0
A. A.	M	48	Sodium nitrite	120 mg. P.O.	226/128	246/146	+11	170/115	98/66†	−44	+
E. C.	F	43	Sodium nitrite	90 mg. P.O.	225/109	210/118	− 2	178/99	88/70‡	−43	+
M. S.	M	22	Sodium nitrite	180 mg. P.O.	118/68	114/64	− 4	96/50	68/40	−26	+
A. P.	M	28	Sodium nitrite	210 mg. P.O.	180/110	174/110	− 2	170/106	176/106	+ 1	0
K. M.	F	51	DHO	0.5 mg. I.V.	218/120	214/128	+ 1	184/94	90/56	−48	+
J. S.	F	45	DHO	0.5 mg. I.V.	194/110	192/102	− 3	174/90	176/99	+ 6	0
E. M.	F	29	DHO	0.5 mg. I.V.	204/104	191/112	+ 3	188/104	108/56	−43	+
J. G.	M	28	DHO	0.5 mg. I.V.	126/84	111/79	− 9	130/82	95/65	−25	0
S. K.	M	43	DHO	0.5 mg. I.V.	120/82	114/78	− 5	114/76	85/62	−23	+
R. K.	M	49	TEAC	300 mg. I.V.	197/120	212/133	+ 9	160/106	127/89	−19	0
E. S.	F	52	TEAC	250 mg. I.V.	255/142	252/149	+ 1	236/146	220/142	− 4	0
J. L.	M	28	TEAC	400 mg. I.V.	140/80	118,78	−12	128/80	100/62	−22	0
G. S.	M	42	C_6	50 mg. I.V.	180/110	160/100	−10	160/100	80/60	−56	+
A. J.	F	49	C_6	50 mg. I.V.	170/120	164/126	0	128/100	90/80	−25	+
T. B.	M	40	C_6	50 mg. I.V.	170/110	155/120	− 2	110/80	85/75	−16	+
L. M.	M	37	C_6	50 mg. I.V.	170/115	160/120	− 1	150/110	75/155	−50	+
S. W.	M	54	Sodium amytal	0.25 gm. I.V.	231/118	220/123	− 2	200/110	192/110	− 6	0
A. L.	M	27	Sodium amytal	0.75 gm. I.V.	130/96	122/92	− 5	128/100	118/100	− 5	0
E. S.	M	61	Sodium amytal	0.5 gm. I.V.	180/104	176/108	0	138/100	124/96	− 8	0
B. G.	M	38	Veratrone	0.8 cc. I.M.	210/128	204/124	− 3	164/96	162/100	+ 1	0
C. B.	F	56	Veratrone	1.0 cc. I.M.	272/180	276/182	+ 1	142/94	146/100	+ 4	0
J. Mc.	M	52	Vertavis	40 CRAW V.PO.	265/160	266/165	+ 1	155/117	145/131	+ 2	0
E. S.	F	52	Vertavis	40 CRAW V.PO.	255/142	252/149	+ 1	200/112	196/114	− 1	0

* "Mean" arterial pressure $= \frac{\text{systolic} + \text{diastolic pressure}}{2}$.

† Collapse occurred $2\frac{3}{4}$ minutes after application of congesting pressure.

‡ Collapse occurred two minutes after application of congesting pressure.

TABLE II

The amount of blood trapped in the extremities subjected to venous congestion before and after sodium nitrite, dihydroergocornine and hexamethonium

Subject	Control			After drug			
	Volume of blood in trunk, head and arm	Total blood volume	Blood trapped in congested limbs	Drug dose and route	Volume of blood in trunk, head and arm	Total blood volume	Blood trapped in congested limbs
	cc.	*cc.*	*cc.*		*cc.*	*cc.*	*cc.*
J. G.*	4,700	6,350	1,650	DHO 0.5 mg. I.V.	4,860	6,400	1,540
S. K.*	4,150	5,480	1,330	DHO 0.5 mg. I.V.	4,070	5,460	1,390
M. S.*	4,040	5,075	1,025	Sodium nitrite 210 mg. P.O.	4,055	5,020	965
R. M.*	2,990	4,230	1,240	Sodium nitrite 180 mg. P.O.	3,010	4,280	1,270
I. P.	5,540	6,660	1,120	Hexamethonium 50 mg. I.V.	5,450	6,540	1,090

* Data on the changes in arterial pressure given in Table I.

No significant reductions of arterial pressure occurred during congestion of the extremities of three patients given 0.25 to 0.75 gm. of sodium amytal intravenously, two subjects given 0.8 or 1.0 cc. of Veratrone (tincture of veratrum viride) intramuscularly and two patients given 40 Craw units of Vertavis (whole powdered root and rhizomes of veratrum viride) orally. In all of the latter cases significant reductions of basal arterial pressure had occurred following the administration of veratrum viride.

The signs and symptoms related to the collapse state induced by sodium nitrite and the "sympatholytic" agents during congestion of the extremities resembled those observed in other types of acute hypotension such as carotid sinus and vaso-vagal syncope, or postural hypotension. The pulse pressure became small and bradycardia frequently developed. Pallor, yawning, sweating and vomiting sometimes occurred. The patients experienced faintness, giddiness and at times nausea.

For example, patient E. C. (Table I) who received 180 mg. of sodium nitrite by mouth 30 minutes prior to congestion exhibited a marked reduction of arterial pressure from 178/100 to 88/70 mm. Hg within three minutes after the venous tourniquets had been applied. Accompanying the hypotensive response there was profuse sweating, pallor and bradycardia. The patient complained of faintness and nausea. Release of the congesting pressure at this time and tilting the patient into a five degree head-down position failed to relieve her symptoms. The arterial pressure continued to fall and one minute after releasing the pressure in the cuffs it was 48/33 mm. Hg and the heart rate was 58 per minute. Four minutes after releasing the congesting pressure the arterial pressure had risen to 110/78 mm. Hg and the heart rate to 72 per minute at which time the patient felt improved.

The amount of blood trapped in the congested limbs

In one hypertensive and four normotensive subjects the amount of blood trapped in the congested limbs was determined by the dye method of Ebert and Stead (3). In the control period the amount of blood present in both lower extremities and one upper extremity during congestion varied between 1,025 and 1,650 cc. with a mean of 1,273 cc. (Table II, Figure 1). There were no significant changes in arterial pressure. Two subjects received sodium nitrite, two were given DHO and one received hexamethonium. During the congestion period following these drugs all of the subjects exhibited significant reductions of arterial pressure and three developed marked hypotension and symptoms of collapse (Table II). The latter occurred two to

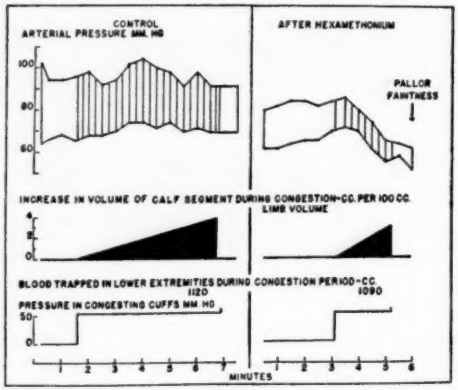

FIG. 1. CHART OF ARTERIAL PRESSURE, INCREASE IN VOLUME OF CALF SEGMENT, AMOUNT OF BLOOD TRAPPED IN THE EXTREMITIES AND CONGESTING CUFF PRESSURES BEFORE AND AFTER INTRAVENOUS HEXAMETHONIUM (50 mg.) IN SUBJECT I.P., NORMAL MALE, AGED 28

In the control period the arterial pressure was well maintained during venous congestion of the extremities for 5¼ minutes. Following hexamethonium and 2¼ minutes after the venous tourniquets had been applied, hypotension and collapse developed. However, the amount of blood trapped in the congested extremities was less and the increase in volume of the calf segment was not as great as in the control period.

four minutes after the beginning of congestion. This necessitated application of occluding pressure prior to the end of the five minute congestion period. This was done in order to prevent blood from either entering or leaving the limbs while the patient was tilted into a head-down position and allowed to recover during the time that the blood volume determinations were being completed.

The amount of blood present in the congested limbs during the experimental period varied between 965 and 1,540 cc. (mean 1,263 cc.). In two subjects there was a slight increase in the amount of blood trapped in the limbs in the post-drug as compared to the pre-drug congestion period and in three cases a slight decrease. In no case did the pre-and post-drug values vary from each other by more than 12 per cent.

Changes in calf volume during venous congestion of the extremities

In order to corroborate these observations indicating that no more blood is trapped in the limbs during venous congestion after as compared with before the administration of agents which induce "congestion collapse," the volume changes in the calf were measured using a plethysmograph. Hexamethonium was administered to all of the subjects.

In the control period the increase in volume of the calf segment during four and one-half to six and one-half minutes of venous congestion varied between 2.4 to 5.2 cc. per 100 cc. of limb volume (mean 3.8 cc.) (Table III, Figure 1). Hypotension did not occur in any of these subjects.

After hexamethonium five of the six subjects developed severe hypotension during periods of congestion of one and one-half to four minutes. The increase in calf volume varied between 2.0 and 3.6 cc. (mean 2.7 cc.). Subject P. G. did not develop severe hypotension during application of venous tourniquets for five and one-half minutes. The *rate of increase* in calf volume during congestion usually was greater after as compared to before hexamethonium (Figure 1). However, in

TABLE III

The increase in volume of the calf segment during application of venous tourniquets before and after the administration of hexamethonium

Subject	Control			After hexamethonium (50 mg. I.V.)		
	Increase in volume of calf segment	Duration of venous congestion	Development of hypotension	Increase in volume of calf segment	Duration of venous congestion	Development of hypotension
	cc. per 100 cc. limb volume	*minutes*		*cc. per 100 cc. limb volume*	*minutes*	
A. G.	3.0	5¾	0	2.8	1¾	+
I. P.	4.0	5½	0	3.0	2¼	+
A. H.	5.2	6¼	0	2.3	2½	+
P. G.	2.8	4⅓	0	5.0	5½	0
J. D.	3.0	5	0	3.6	4	+
L. B.	2.4	6½	0	2.0	1½	+

TABLE IV

The effect of dihydroergocornine (DHO), hexamethonium (C_6), and sodium nitrite on the arterial pressure and heart rate following venesection and subsequent return of the removed blood to the circulation

Pt.	Drug, dose and route	Before drug		After drug and before venesection		During venesection				During return of removed blood			
		Arterial pressure	Heart rate	Arterial pressure	Heart rate	Blood removed	Time from beginning of venesection	Arterial pressure	Heart rate	Blood returned	Time from beginning of return	Arterial pressure	Heart rate
		mm. Hg	*per min.*	*mm. Hg*	*per min.*	*cc.*	*minutes*	*mm. Hg*	*per min.*	*cc.*	*minutes*	*mm. Hg*	*per min.*
L. H.	C_6 50 mg. I.V.	170/100	82	140/80	86	100	2	128/80	92	100	2	130/70	81
						200	3	120/75	92	200	8	140/80	80
						300	4	115/75	100	300	16	150/85	80
						400	8	105/70	100	400	21	172/94	80
						500	12	98/60	80	500	26	180/96	82
J. B.	C_6 50 mg. I.V.	220/120	88	140/90	84	100	1	120/80	84	100	1	90/65	84
						200	2	120/85	80	200	2	100/70	80
						300	5	115/75	80	350	4	130/90	80
						400	6	98/70	88	450	7	135/95	76
						500	8	80/65*	80	500	9	140/100	76
M. D.	DHO 0.5 mg. I.V.	130/100	84	130/100	84	100	2	115/85	80	100	3	110/80	88
						200	4	110/85	92	200	6	105/80	84
						300	7	108/82	96	300	7	100/85	80
						400	12	100/80	96	400	9	110/85	80
						500	15	96/72	104	500	12	110/90	72
M. C.	DHO 0.5 mg. I.V.	220/160	80	220/140	80	100	2	190/120	80	125	4	160/110	76
						200	4	140/105	76	250	9	180/120	76
						250	5	120/90*	80				
E. A.	$NaNO_2$ 210 mg. P.O.	122/82	94	122/82	96	100	2	108/84					
						200	5	110/82					
						300	9	108/78					
						400	17	105/72					
E. R.	$NaNO_2$ 240 mg. P.O.	240/140	60	180/120	84	100	1	180/120	92	120	2	135/95	80
						200	4	150/100	92	200	3	145/100	76
						300	7	150/110	96	300	7	145/110	80
						400	10	160/100	104	400	11	160/120	72
						525	15	125/85	84	525	15	160/120	72
W. O.	$NaNO_2$ 120 mg. P.O.	190/115	80	195/112	80	150	1	165/110	84	150	2	160/108	84
						300	2	155/110	88	400	4	150/110	80
						400	4	155/110	88	500	6	160/110	82
						500	6	155/110	88				

* Yawning, pallor, feels faint.

four of the five patients who developed hypotension during the post-drug congestion period marked reduction in arterial pressure occurred before the *total* increase in calf volume was as great as that observed at the end of the pre-drug congestion period.

The estimation of the amount of blood trapped in the congested limbs as determined by the plethysmographic method agrees approximately with that obtained by the dye method. Previous studies estimate that about 750 cc. of blood are normally present in the lower extremities (3). The average amount of blood found in the congested extremities was 1,270 cc. Thus, the excess of blood pooled out was approximately 1,270 − 750 or 520 cc. Using the plethysmographic method the average increase in calf volume during the control period was approximately 5.6 cc. per 100 cc. of limb volume. If the volume of both lower extremities is estimated to be approximately 10 liters the amount of blood pooled out would be 5.6 × 100 or 560 cc. It should be emphasized that this is a crude estimation since the plethysmograph measures the volume change in only one segment of the limb which may represent a selective rather than a representative portion of the vascular bed being congested.

Effects of certain hypotensive drugs on the arterial pressure during venesection

Seven patients, of whom five were hypertensive, were bled rapidly from an antecubital vein with removal of 250 to 525 cc. of blood (Table IV). Two patients received 0.5 mg. of dihydroergocornine intravenously, two were given 50 mg. of hexamethonium by the same route and three received 120 to 240 mg. of sodium nitrite orally. All of these patients exhibited reductions of average arterial pressure varying from − 10 to − 42 per cent; in five patients the reduction exceeded − 24 per cent. Two patients developed signs of collapse after withdrawal of 250 and 500 cc. of blood, respectively.

The arterial pressure did not fall precipitously at any point in the bleeding period. Instead there usually was a stepwise decrease with each 100 cc. of blood removed (Table IV and Figure 2). Similarly on returning the blood to the circulation the increase in arterial pressure paralleled the rate at which the blood was reinfused. Thus, there was no evidence of a critical amount of blood loss as in untreated subjects beyond which "compensation" fails and the arterial pressure falls suddenly.

As a control the arterial pressures of eight blood donors were measured during venesection. No pretreatment with hypotensive agents had been given. The removal of 500 cc. of blood at a similar rate from each of the donors resulted in a decrease of average arterial pressure of − 8 to + 2 per cent (average − 3 per cent).

Absence of congestion collapse after hypotensive drugs in partially sympathectomized subjects

Eight hypertensive patients who had undergone either a lumbodorsal, or a transthoracic splanchnicectomy four months to five years previously were studied. Two patients received sodium nitrite in doses of 150 to 180 mg. orally, one received 0.3 mg. of the mixed dihydrogenated alkaloids of ergot (CCK) intravenously and five were given 50 mg. of C_6 intravenously (Table V). In only two subjects were significant decreases in average arterial pressure observed (− 11 and − 22 per cent, respectively) during the post drug con-

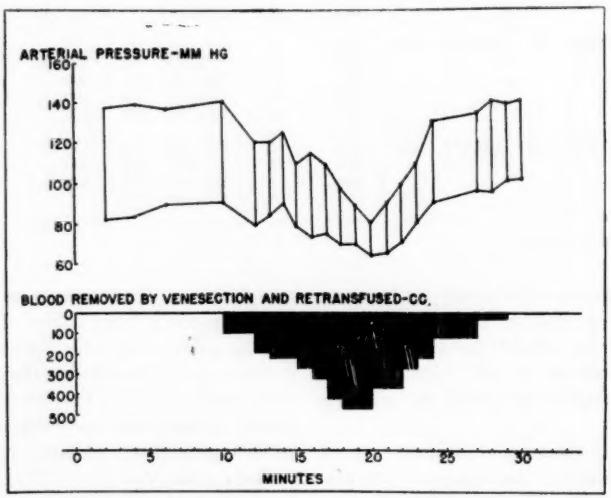

FIG. 2. CHART OF ARTERIAL PRESSURE AND THE AMOUNT OF BLOOD REMOVED BY VENESECTION AND RETRANSFUSED IN PATIENT J. B., FEMALE, AGED 48

Hexamethonium (50 mg.) was given intravenously 30 minutes prior to beginning the venesection. Yawning, pallor and a feeling of faintness developed when the blood pressure fell to its lowest point.

TABLE V

The effect of sodium nitrite, C_6 and CCK on the arterial pressure during congestion of the extremities of sympathectomized patients

Pt.	Sex	Age	Drug	Dose and route	Control			Post-drug period			
					Arterial pressure		Change mean* arterial pressure	Arterial pressure		Change mean arterial pressure	Collapse
					Basal	After five min. of congestion		Basal	After five min. of congestion		
					mm. Hg	*mm. Hg*	*per cent*	*mm. Hg*	*mm. Hg*	*per cent*	
M. C.	F	44	Sodium nitrite	150 mg. P.O.	164/100	159/104	0	156/101	160/105	+ 3	0
P. D.	M	38	Sodium nitrite	180 mg. P.O.	170/110	168/108	−2	146/94	134/90	− 7	0
H. F.	M	36	CCK	0.3 mg. I.V.	212/128	215/135	+3	225/135	220/140	0	0
H. M.	F	43	C_6	50 mg. I.V.	210/125	200/130	−1	150/90	160/95	+ 6	0
M. D.	F	44	C_6	50 mg. I.V.	190/120	185/120	−1	140/100	130/100	− 4	0
D. H.	F	35	C_6	50 mg. I.V.	220/140	205/140	−5	140/120	140/120	0	0
C. M.	M	47	C_6	50 mg. I.V.	210/140	210/138	−1	170/125	140/120	−11	0
P. D.	M	38	C_6	50 mg. I.V.	164/110	160/108	−2	124/95	90/60	−22	0

* "Mean" arterial pressure = $\frac{\text{systolic} + \text{diastolic pressure}}{2}$.

gestion period. Neither of these subjects developed signs or symptoms of collapse. Six of the eight had significant reductions of basal arterial pressure following the hypotensive agents and all of these exhibited increased postural hypotension.

DISCUSSION

The development of collapse during venous congestion of the extremities has been observed in normal subjects (3) and in patients who have suffered recent blood loss (9). Our observations indicated, however, that patients given certain hypotensive agents are unusually susceptible to the development of collapse produced by venous tourniquets and/or hemorrhage. The type of hypotensive agents producing this phenomenon were those which also cause postural hypotension (10, 11), that is, sodium nitrite, hexamethonium, tetraethylammonium and the dihydrogenated alkaloids of ergot; whereas hypotensive drugs such as veratrum viride and sodium amytal which do not ordinarily cause postural hypotension also did not induce congestion collapse.

The increased susceptibility to collapse during venous congestion following sodium nitrite and the sympatholytic agents conceivably might be due to one of several mechanisms. Ebert and Stead have demonstrated that considerable amounts of blood may be trapped out of the central circulation during venous congestion of the limbs (3). They showed that if venous tourniquets were applied for ten minutes or longer almost 30 per cent of the total blood volume may be present in the congested extremities resulting in collapse even in normal untreated subjects. It should be noted that the period of venous congestion had to be longer than five minutes to produce collapse in these untreated subjects. Since, in the present experiments with sodium nitrite and the "sympatholytic agents" hypotension appeared early in the congestion period it seemed possible that these agents induced greater pooling of blood in the congested extremities. This might have resulted from a reduction of "post-arteriolar tone" in the congested limbs produced by these drugs. The increased vascular capacity would thus produce critical loss of blood volume from the central circulation. However, this possibility was disproven by actual measurement of blood volume trapped in the limbs which demonstrated that despite the appearance of hypotension no more blood was trapped out during the post-drug as compared to the control congestion periods.

Plethysmographic measurement of the increase in volume of the calf during congestion also failed to reveal a greater increase in calf volume after as compared with before administration of these agents. In several instances the *rate* of increase in

calf volume was greater following the drug suggesting a decrease in arteriolar and/or post-arteriolar tone (or a failure of reflex arteriolar constriction in the limb). However, marked hypotension occurred before the *total* volume increase was as great as that achieved at the end of five minutes of congestion in the control period. Thus, the observed changes in calf volume provided additional confirmatory data that the collapse was not associated with increased amounts of blood trapped in the limbs.

The increased rate of blood loss to the extremities following these hypotensive agents may have contributed somewhat to the collapse reaction by producing a rapid decrease in venous return and hence of cardiac output. However, the venesection experiments demonstrated that increased rate of blood loss was not the major cause of the hypotensive reaction. Figure 2 shows that the arterial pressure fell despite gradual removal of 500 cc. of blood over a period of eight minutes, whereas a similar rate of removal in the control subjects of these venesection experiments had no significant effect on the arterial pressure.

Another possible explanation for the increased susceptibility to congestion collapse was that the hypotension was due to failure of homeostatic vasoconstriction either arteriolar, post-arteriolar or both, in areas other than in the congested limbs (12). The vascular system in essence is composed of a pump, an arterial conduit leading to a capillary reservoir and a return or venous conduit. The capillary reservoir which has a huge capacity in comparison to the total blood volume is capable of considerable variation in volume. If the reservoir were to enlarge to its full capacity blood would be lost from the large conduits, whereas if it became smaller an excessive amount of blood would be forced into the central circulation. If this closed system were static, blood loss would produce a fall in pressure throughout and a diminution of venous return. However, since the normal vascular system is dynamic, the capillary reservoir is capable within certain limits of reducing its capacity in proportion to the degree of blood loss. Thus, the venous conduit remains filled and venous pressure and return are maintained. Certain hypotensive agents by doing away with the ability to reduce the volume of the capillary beds convert the vasculature into a more static system with the result that during blood loss the pressure falls throughout and venous return fails.

If the collapse were due to failure of compensatory reactions to minor degrees of blood loss from the central circulation it would be expected that moderate blood loss by any route including venesection would produce a similar hypotensive reaction. After the administration of sodium nitrite, the mixed dihydrogenated alkaloids of ergot or hexamethonium, hypotension and collapse did occur following blood loss by venesection in amounts insufficient to induce significant hypotension in untreated individuals. These venesection experiments, therefore, provided direct evidence that the collapse during the post-drug congestion period was due to failure of compensatory vasoconstriction in the presence of relatively small amounts of blood loss.

The present data are not inconsistent with previous observations that sodium nitrite reduces "venous" (post-arteriolar) tone but does not prevent sympathetic vasoconstrictor reflexes involving the arterioles (10, 11). In the prior studies graded congesting pressures of 10 to 50 mm. Hg were applied for brief periods of only a few seconds to demonstrate the reduction in post-arteriolar tone (10). However, it must be emphasized that the congesting pressures of 60 to 100 mm. Hg used in the present experiments are far higher than those normally present in the capillaries and venules (13). When such pressures are applied for as long as five minutes maximum filling of the post-arteriolar vascular bed must occur whether or not sodium nitrite has been given. This accounts for the fact that no more blood was trapped out after as compared to prior to sodium nitrite. Collapse occurs in the post-nitrite congestion period not because of a greater total amount of blood pooled in the limbs but rather from failure of a compensatory reduction in the volume of the post-arteriolar vascular bed in areas other than in the congested limbs. Although this action of sodium nitrite is not definitely clarified the available data imply that while the drug does not prevent arteriolar constriction it must have some paralytic action on the post- arteriolar blood vessels (10). In this respect it differs from the sympatholytic agents which markedly inhibit reflex arteriolar constriction (11).

Perhaps of greater significance was the observation that withdrawal of a relatively small amount of blood by venesection, that is, 100 cc. or 2 to 3 per cent of the total blood volume, resulted in a perceptible decrement in arterial pressure after premedication with a hypotensive drug. This was particularly apparent after hexamethonium, a drug which apparently produces relatively complete sympathetic blockade in man (14). Such observations provide an index of the sensitivity of the homeostatic regulators of the circulation. These data suggest that in the normal, untreated individual losses of less than 5 per cent of the total blood volume stimulate compensatory decreases in the capacity of the vascular tree.

Following administration of adequate doses of the sympatholytic agents the subsequent level of arterial pressure depended in large measure on the position of the patient. A head down posture usually prevented marked hypotension while a slight head up tilt of the body precipitated collapse. In the supine patient following the administration of C_6 marked reductions in arterial pressure could be induced either by tilting, venous tourniquets or moderate degree of blood loss by venesection. These observations are consistent with the concept that the sympathetic vasoconstrictor nerves and associated medullary vasomotor centers are primarily concerned with the rapid reflex adjustment of the capacity of the vascular system, toward regulation of arterial pressure under stress, rather than in the primary regulation of the "basal" level of blood pressure.

The resistance of partially sympathectomized subjects to collapse during venous congestion appears most likely to be related to the mechanisms which result in a gradual restoration of vascular "tone" in denervated areas. This resumption of tone is seen in the disappearance of increased blood flow in the forearm (15), in the hand (16), and in the hepatic-portal circuit (17) after regional sympathectomy as well as the gradual diminution of postural hypotension in patients who have undergone extensive splanchnicectomy (18). The mechanism whereby vascular tone is regained is unknown (19), but apparently, as evidenced by the present experiments, it results in attainment of at least partial homeostasis without dependence on local sympathetic vasoconstrictor reflexes.

SUMMARY AND CONCLUSIONS

1. The prior administration of sodium nitrite, the dihydrogenated alkaloids of ergot, hexamethonium, and to a lesser extent tetraethylammonium to normotensive or hypertensive patients resulted in a state of increased susceptibility to the development of hypotension and collapse when the limbs were congested with venous tourniquets.

2. Veratrum viride and sodium amytal did not produce this effect.

3. Measurement of the volume of blood trapped in the limbs (dye method) and the increase in volume of the calf (plethysmograph) indicated that the hypotension and collapse were not due to pooling of excessive amounts of blood in the congested limbs.

4. The hypotension and collapse were due to failure of compensatory vasoconstriction in other areas than in the congested extremities following moderate blood loss. This was demonstrated by the observation that after the administration of sodium nitrite, the mixed dihydrogenated alkaloids of ergot or hexamethonium hypotension frequently with collapse occurred during venesections of only 250 to 525 cc. of blood.

5. After these drugs loss of as little as 2 to 4 per cent of the total blood volume frequently resulted in perceptible decrements of arterial pressure. Such observations illustrate the extreme sensitivity of the homeostatic vasoconstrictor mechanisms in the normal (untreated) individual.

6. Patients who had previously undergone lumbodorsal or transthoracic sympathectomy were more resistant to hypotension during the post-drug congestion period than were non-sympathectomized subjects. This resistance probably was related to the well known return of independent vascular tone in sympathectomized areas.

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EFFECTS OF ADRENOCORTICOTROPIC HORMONE AND CORTISONE IN PATIENTS WITH TUBERCULOSIS [1, 2]

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INTRODUCTION

In the early reports on the administration of adrenocorticotropic hormone and cortisone to patients with various disease states, there were described a number of effects, the reproduction of which might conceivably alter the course of infections in man. Among these effects were defervescence, decrease in edema and inflammation, change in antibody production, and alteration in the state of hypersensitivity to bacterial products. Clinical evidence of such effects was provided by Finland, Kass and Ingbar (1) who showed that adrenocorticotropic hormone may effect striking alterations in the course of pneumococcal and primary atypical pneumonia. Moreover, equally striking changes were observed by Freeman and his coworkers in two patients with pulmonary tuberculosis (2). The modification of response to infection, by an agent which presumably acts solely upon the host, thus presents an opportunity for further evaluation of the participation of host mechanisms in tuberculous disease.

There are relatively few forms of tuberculosis in humans appropriate for such a study. A serious form of progressive disease, with a reasonably predictable course, is necessary. It is desirable to avoid reliance upon chest roentgenograms as the sole means of evaluating changes in a tuberculous lesion. In this situation, the extent of viable tissue within a lesion must be determined by a procedure which is highly subjective before proper evaluation of subsequent changes within the lesion can be accomplished. Although the accuracy of the method is increased by having the roentgenograms interpreted independently by several competent observers, the interpretations frequently do not reflect the course of the disease as judged by other criteria (3). Such is not the case, however, when a lesion is available in which changes occurring after the introduction of a variable can be easily observed by more direct visualization. For these reasons, a special effort was made to include patients with active laryngeal tuberculosis in this study.

COMPOSITION OF CLINICAL SERIES AND METHODS OF STUDY

Seven patients with far advanced pulmonary tuberculosis were selected for study. Four of the seven had active laryngeal tuberculosis with lesions of the larynx which could be readily visualized by indirect laryngoscopy. A fifth patient had tuberculous empyema on the right side and was considered to be in a terminal phase of her illness. The strains of *M. tuberculosis* isolated in cultures of specimens of sputum from four of the seven patients were highly resistant to dihydrostreptomycin *in vitro*.

The course of tuberculous illness in each patient was evaluated prior to hormone administration from the available clinical, roentgenographic, bacteriologic and immunologic data. Whenever possible, a preliminary observation period of seven to ten days was undertaken. Each patient then received 100 mgm. of hormone intramuscularly in four equally divided doses at six hour intervals for an initial ten day period. Four of the patients received adrenocorticotropic hormone [4] and three received cortisone.[4]

Throughout the study all patients were maintained at rest in bed. They received diets of their own selection and an estimate was made of actual caloric and protein intake. The pulse rate and rectal temperature were recorded at two hour intervals. In addition to customary bedside

[1] Presented in part before The American Society for Clinical Investigation, Atlantic City, N. J., May 1, 1950.

[2] This study was supported in part by grants from The Division of Research Grants and Fellowships, National Institutes of Health, United States Public Health Service; the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.; Chas. Pfizer & Company, Brooklyn.

[3] Post-doctorate Research Fellow, National Institutes of Health, U. S. Public Health Service.

[4] The adrenocorticotropic hormone used in this investigation was obtained through the courtesy of Dr. John Mote, Armour and Co., Chicago, Ill. The cortisone was obtained through the courtesy of Dr. James M. Carlisle, Merck and Co., Rahway, N. J.

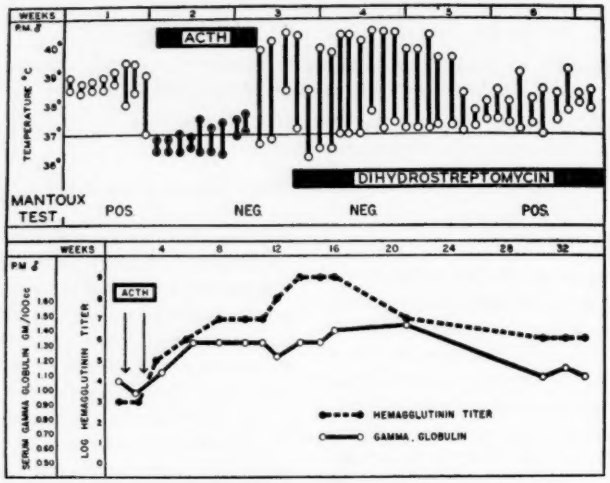


FIG. 1. PATIENT P. M., AGE 49 YRS.

In the upper portion of the chart are shown the daily range of temperature and the results of tests for cutaneous hypersensitivity to tuberculoprotein. Note the rapid defervescence following the start of ACTH administration, and the abrupt return of fever with wide fluctuations after stopping ACTH. The return of cutaneous hypersensitivity to tuberculoprotein coincides with the fall in fever while under treatment with dihydrostreptomycin. In the lower portion of the chart are shown the alterations in the values obtained for concentrations of serum gamma globulin and in the titer of hemagglutinating antibodies.

observations, indirect laryngoscopy was performed on each patient with laryngitis. Drawings were made of the laryngeal lesions as seen in the laryngeal mirror, at intervals determined by change in the appearance of the lesions. Serial chest roentgenograms, using a standardized technique, were obtained at 72 hour intervals.

Twenty-four hour aliquots of continuous sputum collections were measured for volume and the character of the specimen noted. The aliquots were then digested, centrifuged and direct smears were made of the concentrate. Duplicate smears were stained with carbol fuchsin by the Ziehl-Nielsen technique and with Sudan black B (4). Petragnani and oleic acid-albumin media were used for cultivation of the *M. tuberculosis* from the concentrated specimens of sputum. The colonial morphology of the bacilli growing on oleic acid-albumin medium was examined for the characteristics known to be associated with various degrees of virulence (5). Organisms obtained from each patient at weekly intervals were grown in Tween[5]-albumin medium and were tested for sensitivity to dihydrostreptomycin.

Observations on the cutaneous hypersensitivity to tuberculin were made using an amount of tuberculoprotein, standardized individually for each patient, sufficient to obtain an unequivocally positive reaction. An initial reaction of more than 10 mm. in diameter showing erythema and definite edema was obtained in five patients using 0.00002 mgm. of Tuberculin, Purified Protein Derivative[6] (First Test Strength). In one patient (P. M.), 0.005 mgm. of Tuberculin, Purified Protein Derivative (Second Test Strength) was required to produce a reaction of this magnitude. The reactions were examined and the outlines traced on cellophane at 48 and 96 hours after intracutaneous injection of the forearm.

Specimens of serum were obtained at frequent intervals from each patient for estimation of tuberculin hemagglutinating antibody titer as described by Middlebrook and Dubos (6) and the concentration of serum gamma globulin by the method of Kunkel (7). Bone marrow examinations with particular reference to the plasma cell count were done as described by Good and Campbell (8). Other laboratory examinations were made in each patient and gave results comparable to those reported in other studies with these compounds.

[5] "Tween 80" is the trade name of the polyoxyethylene derivative of sorbitan monooleate, obtained from the Atlas Powder Company, Wilmington, Del.

[6] The Tuberculin, Purified Protein Derivative used in this investigation was obtained from Parke, Davis and Co., Detroit, Mich.

RESULTS

Course of illness: In five patients the onset of hormone administration marked the inception of a series of profound changes, the first of which were evident as early as six to eight hours after the initial dose. In all patients, a strikingly rapid disappearance of the signs and symptoms of acute illness occurred and persisted throughout the period of hormone administration. Following the cessation of hormone administration, however, a quick return to the previous state of illness was observed.

In three of the four febrile patients defervescence was complete within six hours of the start of hormone administration (Figures 1–3). This process required 72 hours for completion in one patient (Figure 4). All patients remained essentially afebrile until the cessation of hormone administration. Defervescence was accompanied by a feeling of increased strength and desire for physical activity. Appetite increased markedly with concurrent rise in the caloric and protein intake.

In the four patients with laryngitis improvement in phonation and disappearance of dysphagia were evident by the end of the initial 48 hour period of hormone administration. Thereafter, a progressive increase in the volume of the voice and improvement in its quality ensued. Discomfort from dyspnea and cough was markedly relieved although the volume of the sputum was not significantly decreased. All of the patients experienced some degree of a sense of well-being which in some amounted to euphoria.

The cessation of hormone administration was followed by abrupt return of the signs and symptoms of acute illness. The manifestations of acute illness immediately following the end of hormone administration were at least equal in severity to those present before hormone administration and

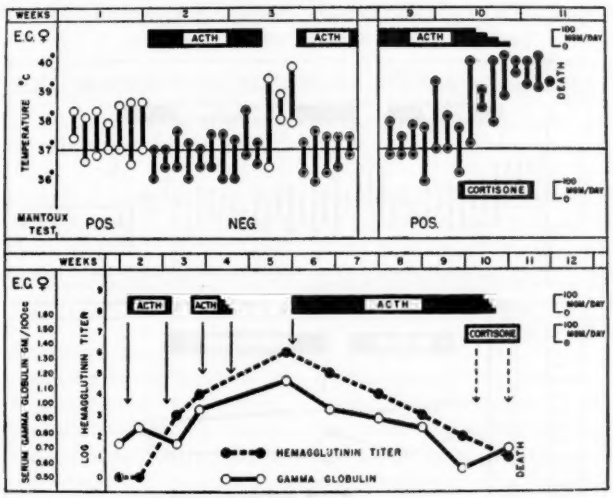


FIG. 2. PATIENT E. C., AGE 21 YRS.

Note the rapid defervescence and abrupt return of fever at the onset and end, respectively, of the initial course of ACTH administration. Following reinstitution of ACTH administration prompt defervescence occurred. Escape from the controlling effects of ACTH occurred later with return of fever and cutaneous hypersensitivity to tuberculoprotein despite the addition of cortisone. In the lower panel are shown the rises in serum gamma globulin values and in titer of tuberculin hemagglutinating antibodies following the initial course of administration of ACTH. Note the progressive decline of both serum gamma globulin values and hemagglutinin titer during prolonged ACTH administration.

in four patients (P. M., E. C., S. H., and T. B.), were more severe. Fever occurred within 18 hours after the last dose of adrenocorticotropic hormone. Following the cessation of cortisone administration, however, fever occurred on the second and sixth day (Figures 4 and 5) or was not a prominent feature in the post-hormone course (Figure 6).

The fever following the end of hormone administration was self-limited in two patients who were essentially afebrile prior to hormone administration (Figures 5 and 7). In one patient (Figure 1) despite the institution of dihydrostreptomycin therapy soon after stopping adrenocorticotropic hormone, fever continued for four weeks. It may be noted here that in this patient, subsidence of fever coincided with the return of cutaneous hypersensitivity to tuberculoprotein. Significant defervescence was not observed during dihydrostreptomycin administration in another patient (Figure 4). Subsequently, the strain of *M. tuberculosis* isolated from cultures of the sputum of this patient was found to be resistant *in vitro* to dihydrostreptomycin.

Laryngeal lesions: Observations of the changes in the laryngeal lesion was made by daily indirect laryngoscopic examination in the four patients with tuberculous laryngitis. In all patients a similar sequence of changes was observed. The changes noted in the laryngeal lesion of one patient (P. M.) are depicted in Figure 8 and may be considered as characteristic of the changes observed in all patients studied.

In this patient, initial examination of the left side of the larynx demonstrated ulceration with partial replacement by granulation tissue of the lateral portion of the arytenoid tubercle, aryepiglottic fold and true vocal cord, in addition to absence of one-half of the epiglottis. The entire right half of the larynx was edematous with marked inflammation and both true vocal cords were irregular.

Forty-eight hours after the beginning of hormone administration, edema and inflammation had subsided almost completely. In the ulcerated le-

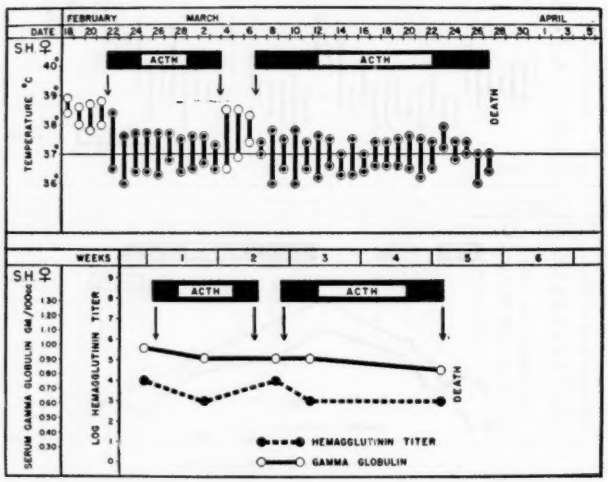


FIG. 3. PATIENT S. H., AGE 36 YRS.

Rapid defervescence following the onset of the initial course of ACTH administration and abrupt return of fever at the end of this period of administration are shown. Upon reinstitution of ACTH administration, defervescence again occurred rapidly. Death ensued during prolonged ACTH administration without return of fever. In this patient no significant alterations occurred in serum gamma globulin values or in titer of hemagglutinating antibodies following the initial ten day course of ACTH or upon prolonged ACTH administration.

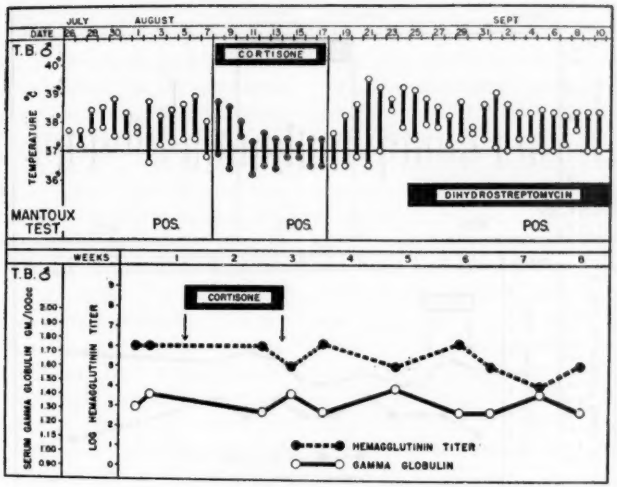


FIG. 4. PATIENT T. B., AGE 40 YRS.

Following the onset of cortisone administration, defervescence required 72 hours for completion. Fever slowly recurred following cessation of cortisone administration and persisted despite dihydrostreptomycin therapy. Subsequently the strain of *M. tuberculosis* isolated from culture of the sputum was found to be resistant to dihydrostreptomycin. Reversal of cutaneous hypersensitivity to tuberculoprotein was not observed in this patient. No significant alterations occurred in serum gamma globulin values or in titer of the tuberculin hemagglutinating antibodies.

sions, a border of epithelium could be visualized progressing from the edges of the ulcerated area as the granulation tissue subsided. By the ninth day of hormone administration, the epidermization had covered approximately 85 per cent of the previously ulcerated area.

Within 48 hours after completion of hormone administration, edema and inflammation had returned although no regression of epithelium was observed. Subsequently, no significant alterations in the appearance of the laryngeal lesion attributable to hormone effect were observed.

Complete healing with scarring occurred following the institution of dihydrostreptomycin therapy in two patients whose sputum contained strains of *M. tuberculosis* found to be sensitive to the drug *in vitro*.

Lung lesions: Serial roentgenograms, interpreted independently by several observers, revealed definite changes in the appearance of the lung lesions. These changes formed a consistent pattern in five of the seven patients. A representative example of the changes is shown in Figure 9 portraying enlarged segments of chest roentgenograms from one of the patients (P. M.).

In general, during the administration of adrenocorticotropic hormone the appearance of lung lesions became more translucent with better delineation of the individual lesions. Within 48 hours after cessation of hormone administration definite increase in the density of the lesions was apparent. No patient in this series has been observed without additional therapeutic measures for a sufficient time beyond this point to evaluate the roentgenographic changes which might result solely from hormone administration.

Dihydrostreptomycin therapy was instituted in this patient (P. M.) and continued for 111 days. The increase in density reached a maximum in the fourth week, the lesions thereafter became more translucent. It is of interest that in this patient the onset of defervescence and the return of cutaneous hypersensitivity to tuberculoprotein also occurred during the fourth week after the end of

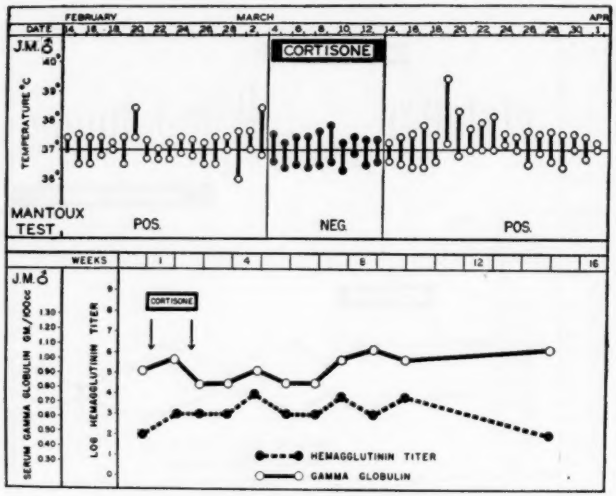


FIG. 5. PATIENT J. M., AGE 51 YRS.

Fever occurred on the sixth day after cessation of cortisone administration and gradually subsided over a 48 hour period. Reversal of cutaneous hypersensitivity to tuberculoprotein is illustrated in the upper panel. No significant alterations in serum gamma globulin values or in titer of tuberculin hemagglutinating antibodies occurred in this patient.

hormone administration. Progressive decrease in the size and density of the roentgenographic appearance of the lung lesions ensued, marked clearing being noted seven months after hormone administration (Figure 9).

Resorption of a small purulent intrapleural exudate was observed in one patient during the administration of adrenocorticotropic hormone. As this patient (S. H.) was in the terminal phase of her illness, prolonged observation after hormone administration was impossible. No well-defined changes in the roentgenographic appearance of the lung lesions occurred in two patients, one receiving adrenocorticotropic hormone (A. R.) and the other cortisone (J. M.).

Roentgenographic evidence of dissemination of the tuberculous process was not obtained in any patient.

Skin hypersensitivity to tuberculoprotein: Repeated observations on the skin hypersensitivity to tuberculoprotein were made. In each of the six patients tested an amount of tuberculin (Purified Protein Derivative) was used sufficient to elicit an unequivocally positive reaction prior to the administration of hormone. Reversal of cutaneous hypersensitivity to this individually standardized amount of tuberculoprotein was observed during hormone administration in three of the six patients tested. This reversal of a previously positive response was maintained for three to four weeks after the cessation of hormone administration in the two patients followed from this standpoint. In three patients (Figures 4, 6 and 7) negative reactions were not obtained.

It is of considerable interest that despite the continued absence of cutaneous hypersensitivity to the test dose of tuberculoprotein the acute manifestations of tuberculous illness reappeared following cessation of hormone administration in the three patients in whom reversal of the tuberculin skin test was obtained.

Serum gamma globulin and tuberculin hemagglutinating antibodies: The substantially elevated serum gamma globulin values initially present in one patient (Figure 7) decreased toward the normal range during and immediately following adrenocorticotropic hormone administration. Subsequently, the values rose, reaching the high level

present initially by the end of four weeks, and rose even further thereafter. Aside from this instance, significant variations in the serum gamma globulin values did not occur during the ten day period of hormone administration. Likewise, the titers of the tuberculin hemagglutinating antibody were not significantly changed during this period.

Within three weeks after completion of hormone administration, however, three patients had significant increases both in the concentration of serum gamma globulin and in the titer of the tuberculin hemagglutinating antibodies (Figures 1, 2 and 6). The rises were sustained in one patient (P. M.) in whom the values reached a maximum between the 14th and 16th weeks and declined thereafter. Although significant increases in both serum components were noted in another patient (R. R.) immediately following cessation of cortisone administration, the evolution of the changes has been observed for only a short period of time. In the third patient (E. C.) reinstitution of adrenocorticotropic hormone at the peak of the rise in serum gamma globulin and hemagglutinating antibody titer was followed by progressive decline toward the original values.

Prolonged hormone administration: Two of the most critically ill patients (E. C. and S. H.) received additional adrenocorticotropic hormone to relieve the extreme prostration which accompanied the return of fever. The strains of *M. tuberculosis* recovered in cultures of the sputum of both were highly resistant to dihydrostreptomycin *in vitro*. Upon reinstitution of 100 mgm. per day of adrenocorticotropic hormone, the onset of defervescence was again rapid and complete and the signs and symptoms of acute illness disappeared promptly.

In one of these patients (E. C.) an unsuccessful attempt was made to decrease the severity of the post-hormone manifestations of illness by gradual reduction of the amount of hormone administered over a four day period. Subsequently, reinstitution of adrenocorticotropic hormone was necessary to alleviate the manifestations of acute illness. During the fourth consecutive week of hormone

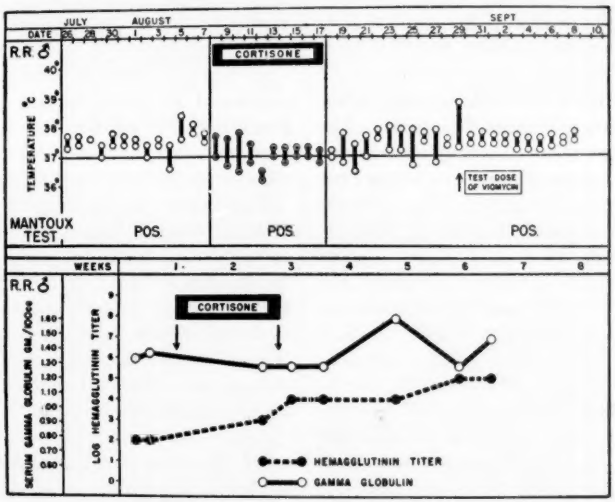


FIG. 6. PATIENT R. R., AGE 25 YRS.

Slight decrease in the daily temperature range during cortisone administration is shown in the upper panel. No post-hormone fever attributable to the cessation of cortisone administration occurred in this patient. Reversal of cutaneous hypersensitivity to tuberculoprotein was not observed. Significant increases in both serum gamma globulin values and in titer of hemagglutinating antibodies immediately following cessation of cortisone administration are shown.

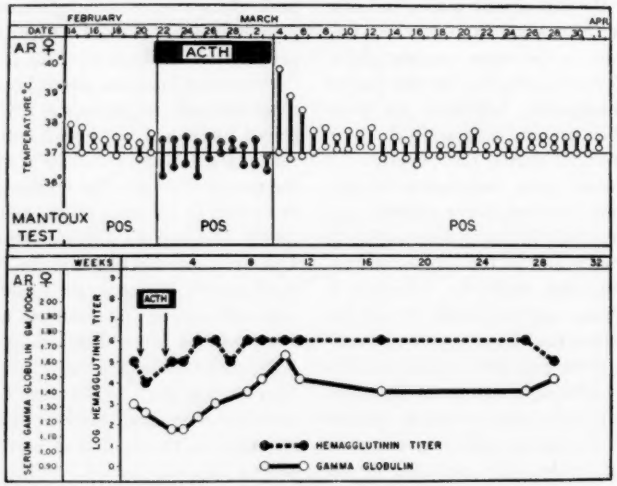


FIG. 7. PATIENT A. R., AGE 39 YRS.

Note the occurrence of fever immediately following cessation of ACTH administration in this patient who was essentially afebrile prior to hormone administration. Reversal of cutaneous hypersensitivity to tuberculoprotein was not observed. Decrease in serum gamma globulin values occurred during and immediately after cessation of ACTH administration, and subsequently rose to values higher than the initial level.

administration, in the ninth week of study, hallucinations and delusions appeared abruptly. The following week fever recurred with gradual return of malaise, cough, and increasing prostration; cutaneous hypersensitivity to the test dose of tuberculoprotein also reappeared (Figure 2). The patient's condition rapidly became worse, despite continuation of adrenocorticotropic hormone and the addition of 100 mgm. per day of cortisone. Gradual reduction and cessation of adrenocorticotropic hormone administration failed to produce evidence of improvement. Cortisone administration was maintained for four days without apparent change in the patient's clinical condition and was discontinued in the fifth consecutive week of hormone administration. Thirty-six hours following cessation of cortisone administration, this patient expired.

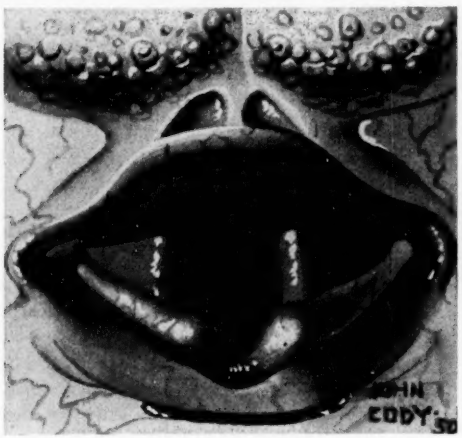
Reinstitution of adrenocorticotropic hormone in another patient (Figure 3) was followed by a succession of events similar to those described above, although less pronounced. Marked symptomatic improvement and defervescence were gradually supplanted by euphoria and general weakness. Death occurred on the 21st consecutive day of hormone administration in the fifth week of study without recurrence of prostration or fever.

Post-mortem examination revealed no evidence of recent dissemination of the tuberculous process or significant alteration in the histopathology of the tuberculous process in either patient. The adrenal cortices of both patients were uniformly thickened and the glands were increased in weight. It is of interest that a small nodule of caseation necrosis was found at the surface of the right adrenal gland in one patient (S. H.).

Subsequent course: Two of the patients (P. M. and J. M.) have shown steady, favorable progress following the institution of dihydrostreptomycin and para-amino-salicylic acid administration. Sputum concentrates and cultures no longer show the presence of *M. tuberculosis*. Marked clearing in the chest roentgenogram of P. M. has occurred as previously noted. The activity of his tuberculous process being considered arrested, J. M. has been discharged from the hospital.

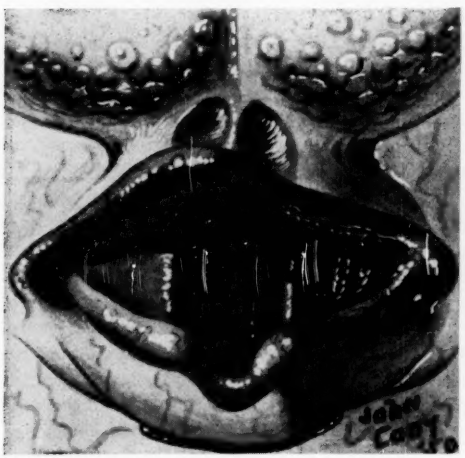
One of the patients (A. R.) whose sputum contained a strain of *M. tuberculosis* found to be resistant to dihydrostreptomycin *in vitro,* has been observed for ten months while receiving pneumoperitoneum therapy and bed rest. Her clinical status, though not conspicuously changed, has shown slight improvement. No significant alteration of her laryngeal lesion has been observed since the changes noted immediately after cessation of adrenocorticotropic hormone administration.

The two remaining patients (T. B. and R. R.) have not been observed for a sufficient period of time following the administration of cortisone to permit final evaluation of the influence of the hormone on the course of the infection. No dele-



a) Normal larynx as seen in a laryngeal mirror.

b) P. M.—Larynx before ACTH.



c) P. M.—Larynx after nine days of ACTH.

d) P. M.—Larynx two days after cessation of ACTH.

FIG. 8. PATIENT P. M. CHANGES IN THE LARYNGEAL LESIONS

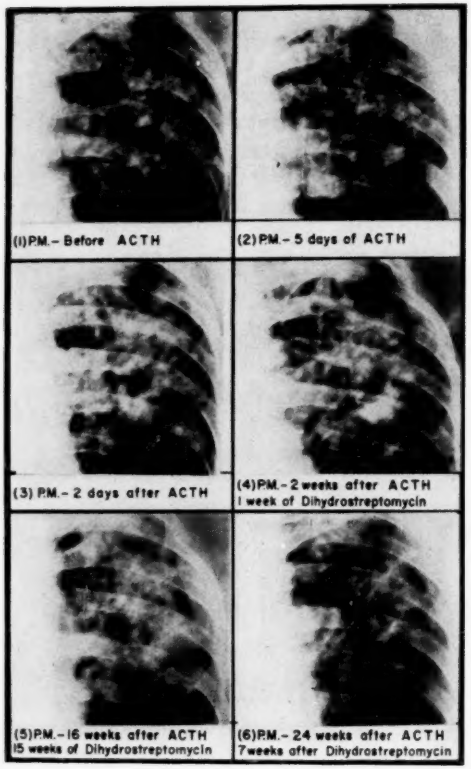

(1)P.M.- Before ACTH
(2)P.M.- 5 days of ACTH
(3) P.M.- 2 days after ACTH
(4)P.M.- 2 weeks after ACTH
1 week of Dihydrostreptomycin
(5)P.M.- 16 weeks after ACTH
15 weeks of Dihydrostreptomycin
(6)P.M.- 24 weeks after ACTH
7 weeks after Dihydrostreptomycin

FIG. 9. PATIENT P. M. CHANGES IN THE ROENTGENOGRAPHIC APPEARANCE OF THE LUNG LESIONS

(*1*) An enlarged segment of the initial chest roentgenogram. (*2*) Note slight decrease in density of the lung lesions. (*3*), (*4*) Note marked increase in density in the appearance of the lung lesions. (*5*), (*6*) Marked clearing of lung lesions after dihydrostreptomycin.

terious effect of cortisone administration, however, has been noted during the four month period of observation.

Tubercle bacilli: Tubercle bacilli were discharged in the sputum of all patients without significant fluctuations in number throughout the period of observation. Retention by the tubercle bacilli of initial staining properties with carbol fuchsin and Sudan black B and of initial *in vitro* sensitivity to dihydrostreptomycin was observed. Colonies grown on oleic acid-albumin attained the morphologic characteristics attributed to virulent organisms by Middlebrook, Dubos, and Pierce (*5*) in all specimens examined. Therefore, no bacteriologic evidence of alteration in the tubercle bacilli isolated from sputum or empyema was obtained.

COMMENT

From the observations reported above, it appears that abrupt and profound changes in the course of tuberculous illness may be brought about by the introduction of hormones which presumably affect only the host.

During the period of administration of these hormones, rapid amelioration and subsequent disappearance of the constitutional manifestations of acute illness were witnessed. Complete defervescence was accompanied by a seemingly improved state wherein increase in strength, appetite, and desire for activity occurred. It is of particular interest to note that the onset of the seemingly improved state was equally evident in two patients (J. M. and A. R.) who were essentially afebrile prior to hormone administration. Improvement in phonation and disappearance of dysphagia was striking as was the recession and subsequent quiescent appearance of the laryngeal lesions. Decrease in density of the lung lesions seen on chest roentgenogram was noted in five patients. Reversal of cutaneous hypersensitivity to an arbitrarily selected amount of tuberculoprotein was observed in three of the six patients tested.

The termination of the state of apparent improvement following the withdrawal of hormone administration was equally evident. The signs and symptoms of acute illness which had been alleviated, reappeared abruptly, and were equal to or even more severe than those present before hormone administration. The return of edema and inflammation in the laryngeal lesions also occurred promptly as did the increase in density of lung lesions seen on chest roentgenograms. The swift return of the manifestations of acute illness contrasted sharply with the relatively slow return of cutaneous hypersensitivity to tuberculoprotein and rise in the titer of the tuberculin hemagglutinating antibodies observed several weeks after cessation of hormone administration.

The abrupt disappearance of the manifestations of illness induced by adrenocorticotropic hormone and cortisone is of considerable interest in regard to the nature of the toxicity of tuberculosis. The

mechanisms by which this infection produces fever and other manifestations of illness are not completely understood. It has been generally believed that the tissue damage and the clinical illness are, in large part, consequences of the interaction of previously sensitized tissue and the absorbed products from tubercle bacilli (*9a*). *In vitro* studies have been demonstrated that cells of the hypersensitive body are damaged and killed by amounts of tuberculoprotein that are harmless to cells in the absence of hypersensitivity (*9b*). Whether the living tubercle bacillus exerts a primary toxic effect upon host tissue is not definitely established (10). Diminution in the manifestations of illness might be expected if, by alteration in the reactivity of host tissue, some degree of desensitization to the products of infection was obtained. Conversely, upon reappearance of the effects of hypersensitization their return would not be surprising.

From the observations reported here, it seems clear that reactivity of host tissues sufficient to cause severe symptoms of tuberculous illness may be present without the presence of a positive Mantoux test. Moreover, distinct symptomatic improvement was observed after the start of hormone therapy in those patients whose Mantoux test remained positive. The inability to utilize clinical quantitation and subsequent correlation of local and systemic tissue hypersensitivity to tuberculoprotein makes speculation on this aspect inadvisable.

The sudden suppression of the clinical evidence of toxicity observed to occur at the start of hormone administration may be compared to the similar state observed in patients with tuberculosis under treatment with streptomycin. Loss of so-called "toxemic" symptoms may occur in both situations and although improvement following the initiation of streptomycin therapy may be observed as early as the third day of therapy, it has not been observed to be as rapid in onset nor does its speed approach that of the changes occurring in the patients reported above. Thus the same phenomenon, sudden suppression of the clinical evidences of toxicity, may be produced on the one hand, by actions exerted principally upon the parasite and on the other, by action exerted solely through the host.

The course of the two patients who expired suggests that the changes effected by a fixed amount of adrenocorticotropic hormone are temporary and cannot be sustained, even with continued administration. Whether modification of the amount of hormone administered, alteration in the frequency of administration, or the combination with other agents will alter this situation is yet to be determined.

SUMMARY

The effects of adrenocorticotropic hormone and cortisone on the course of tuberculous illness has been studied in seven patients with far advanced pulmonary tuberculosis. It was found that abrupt and profound changes in the course of the illness were brought about by administration of these hormones. These changes were in the nature of defervescence, improvements in the sense of well-being, increased strength, improvement in appetite, as well as decrease in the edema and in the amount of exudate in the local lesions of the larynx. In all patients the improved state was temporary and, as might be anticipated, could not be indefinitely maintained by further administration of the hormones.

Observations were also made on the changes in cutaneous hypersensitivity to tuberculoprotein, in concentration of serum gamma globulin and in the titer of tuberculin hemagglutinating antibodies. Reversal of cutaneous hypersensitivity to an arbitrarily selected amount of tuberculoprotein during hormone administration was observed in three of the six patients tested. Within three weeks after completion of hormone administration significant increases both in concentration of serum gamma globulin and in the titer of tuberculin hemagglutinating antibodies were noted in three of the seven patients.

Interpretation of these temporary alterations in the disease state in terms of ultimate effect in such a chronic infection must await further study.

ACKNOWLEDGMENTS

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AGING AS A FACTOR IN THE RENAL HEMODYNAMIC CHANGES INDUCED BY A STANDARDIZED PYROGEN

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The functional and anatomic changes of the kidney associated with increasing age have been demonstrated convincingly. The evaluation of renal function in aged persons has demonstrated a progressive decrease in the urea clearance (1), glomerular filtration rate, effective renal plasma flow, and renal tubular transfer maxima (2, 3). These decreases in function fit well with the anatomic studies which hold that the degree of renal arteriosclerosis present in the aging population is sufficient to account for the decrease in renal parenchyma on a basis of restricted blood supply (4–7).

The anatomic studies also agree that the predominant picture of arterial degeneration is that of intimal thickening (lumen attenuation and occlusion) in the larger arteries (interlobar and interlobular) with progressively less sclerosis in the vessels as the observations extend toward the glomerulus. Even in the age group beyond 80 years, Bell notes that only about 20 per cent of the renal arterioles are involved in the sclerotic process, and this involvement is not severe (7). In his study of the relationship of age to renal atrophy in non-hypertensive individuals free from renal disease, Bell states, "Areas of atrophy are wedge-shaped and are caused by intimal atherosclerosis with occlusion of fairly large branches of the renal artery."

The present study is concerned with an evaluation of the functional status of the renal arterioles in an aging population free of hypertension and demonstrable renal disease. Since it is believed that the increase in renal blood flow following the intravenous administration of a pyrogen is in large part mediated through a decrease in renal arteriolar tonus (8), we have employed a standardized pyrogen test to assay the capacity of the renal arterioles to dilate.

METHODS

Subjects. The 54 male subjects studied were divided into three age groups: young (Y), age range 20–49, mean age 36.6 years, N = 20; middle (M), age range 50–69, mean age 58.8, N = 20; and old (O), age range 70–84, mean age 76.9 years, N = 14. The broad 30-year age span of the young group was so selected because the downward age trend in renal function is not significant until after the age of 50 (2). That this finding applied well to the present group is indicated by the similarity of the mean resting effective renal plasma flows for two subgroups: age 20–34, 609 cc./min./1.73 m.^{2}; age 35–49, 599 cc./min./1.73 m.^{2}

The subjects were patients on the acute and chronic wards and residents of the Infirmary (Home for the Aged) of the Baltimore City Hospitals. All patients studied were ambulatory.

Except as noted, subjects were selected who met the following criteria: *a*) absence of history of physical evidence of renal disease, hypertension, or congestive heart failure, *b*) urinalyses (two or more) negative for protein by the heat and acetic acid coagulation test and for significant microscopic abnormalities, *c*) blood hemoglobin greater than 12 grams per cent, *d*) absence of electrocardiographic evidence of arrhythmia or A–V conduction defect, *e*) blood urea nitrogen within the limits for the different age groups as reported by Lewis and Alving (1), and *f*) basal diastolic blood pressure below 90 mm. Hg. No limits were placed on the systolic blood pressure, which ranged from 100 to 181 mm. Hg. In the old group, three of the subjects, though normotensive at the time of testing, showed cardiac enlargement and previous hypertensive blood pressure readings. Also in the old group an additional two subjects showed signs of congestive failure. However, the mean baseline renal function values and the mean renal hemodynamic response for these individuals were the same as the mean for the other nine subjects in this group.

Additional clinical examinations were made which were not used for exclusion of subjects. Heart size was estimated by x-ray; seven subjects were included who showed cardiac enlargement (cardiac transverse diameter 10–20 per cent in six cases and 32 per cent in one case above normal for their height and weight). Electrocardiographic abnormalities suggestive of myocardial disease

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were noted in 11 subjects, eight of whom were in the old group. Palpable thickening and diminished pulsation of the brachial, radial, dorsal pedal, and posterior tibial arteries were noted. Retinal arteriosclerosis was graded by an ophthalmologist.

Test procedure. Subjects were studied in the post-absorptive state beginning at 8 a.m. Aminopyrine (0.6 gram) was given by mouth at four-hour intervals from 4 p.m. on the day preceding the test until 8 p.m. on the day of the test. Using the renal clearance technique of Goldring and Chasis (9) eleven 20-minute urine collection periods were carried out starting immediately after the intravenous administration of 50,000,000 killed typhoid organisms (0.05 cc. TAB vaccine). Blood samples were obtained through an indwelling femoral artery needle.

The sphygmomanometric blood pressure and the pulse rate by one-minute radial artery counts were determined at the fifth and 15th minute of each period. The average of the two determinations was recorded as the value for the period. Oral temperature was determined hourly during and for six to eight hours after the test.

Chemical methods. Inulin was determined in blood and urine by the method of Harrison (10). PAH was determined in blood and urine by the method of Bratton and Marshall (11) as modified by Smith and associates (12). Blood urea nitrogen was analyzed by the method of Van Slyke (13). Total serum proteins were determined by the micro-Kjeldahl method (14) on 12 of 20 Y group subjects, 15 of 20 M group subjects, and 11 of 14 O group subjects. Hematocrits were determined by the Wintrobe method on the control blood samples drawn from each subject.

Analysis of data. Clearances of inulin and PAH were corrected to 1.73 $m.^2$ of body surface. Renal afferent and efferent hydraulic resistances were calculated according to the formulae of Lamport (15) and were expressed as 10^4 mm. Hg (min./cc.) (body surface $m.^2/1.73$ $m.^2$).

Mean blood pressure was taken as equal to the diastolic pressure plus one-half the pulse pressure. The mean serum protein values for the appropriate age group were used for the calculation of resistances in a few subjects where individual serum protein values were not available and an albumin globulin ratio of 2.2 was assumed for all subjects.

For each hemodynamic measure mean curves of the pyrogen reaction were constructed for each of the three age groups. In addition, to evaluate the significance of differences between age groups, individual response indices were computed as the difference between the baseline (average of the first two periods) and the peak response (average of the two consecutive periods which gave a maximum or minimum for the particular measurement). For the analysis of blood pressure responses, the average of periods X and XI was used instead of the individual maximum responses, because of qualitative differences in the blood pressure responses of the three age groups. The mean differences between age groups in the baseline values and response indices were evaluated for significance by the t test. Only t scores at the 1 per cent level of probability are reported as significant.

The rate of increase in C_{PAH} was estimated as the slope of a straight line drawn by inspection through the points which included the period of rising C_{PAH}. The value for the individual was based on the average of slopes determined independently by two of the authors. The correlation between the two determinations was + 0.98.

RESULTS

Glomerular filtration rate (C_{IN}). The mean baseline values for the C_{IN} in the Y, M, and O groups were 110.4, 88.9, and 62.4 cc./min., respectively (Table I). The course of the mean C_{IN} was similar through the reaction in all three age groups in that there was neither a significant increase nor decrease present (Figure 1). Subjects occasionally showed a rise or fall in C_{IN} which bore no relationship to the degree of their increase in C_{PAH}.

Effective renal plasma flow (C_{PAH}). The mean baseline values for the Y, M, and O groups were 603, 449, and 277 cc./min., respectively (Table I). Baseline C_{PAH} was unrelated to the presence or absence of any of the observed clinical characteristics. Thus, the mean baseline C_{PAH} for subjects with retinal arteriosclerosis was not significantly different from the mean for those in the same age group with no retinal arteriosclerosis. The same was true for subjects with and without peripheral arteriosclerosis, elevation of systolic blood pressure, electrocardiographic abnormalities, or any combination of the observed clinical characteristics.

The group response curves are shown in Figure 1 for the three age groups. In this figure the simultaneity of time of onset, and the rate of C_{PAH} increase are shown. The mean absolute increases in the clearance of PAH for the Y, M, and O groups were 416, 368, and 240 cc./min., respectively. Expressed as per cent of baseline value, the rise in C_{PAH} for the Y, M, and O groups was 71, 86, and 91 per cent, respectively (Table I).

In a further attempt to characterize the increase in C_{PAH} of the different age groups, a slope for the rate of reaction of each individual was obtained as an expression of rate of response per unit of time. The values obtained for the Y, M, and O groups were 5.9, 4.7, and 3.4 cc./min., respectively (Table I). Expressed as per cent of the baseline value, the rise in C_{PAH} was 1.02, 1.10, and 1.35 per cent for the Y, M, and O groups, respectively.

TABLE I

Age differences in the average hemodynamic responses to pyrogen

Measurement	Age groups			Significance of differences*		
	Y 20–49 yrs.	M 50–69 yrs.	O 70–85 yrs.	Y–M	M–O	Y–O
C_{PAH} (cc./1.73 m.²/min.)						
Baseline	603	449	277	S	S	S
Maximum	1019	817	517	S	S	S
Δ	416	368	240	S	S	S
Per cent increase	71	86	91	NS	NS	NS
C_{PAH} rise/min.	5.9	4.7	3.4	NS	PS	S
C_{IN} (cc./1.73 m.²/min.)						
Baseline	110.4	88.9	62.4	S	S	S
FF (C_{IN}/C_{PAH})						
Baseline	.189	.206	.232	NS	NS	S
Minimum	.115	.123	.122	NS	NS	NS
Δ	.074	.083	.110	NS	NS	S
Pulse rate (beats/min.)						
Baseline	67	65	60	NS	PS	S
Maximum	85	80	76	NS	NS	PS
Δ	18	15	16	NS	NS	NS
Systolic blood pressure (mm. Hg)						
Baseline	116	124	136	PS	NS	S
Periods X and XI	114	110	121	NS	NS	NS
Δ	2	14	15	S	NS	S
Diastolic blood pressure (mm. Hg)						
Baseline	77	77	75	NS	NS	NS
Periods X and XI	67	63	64	NS	NS	NS
Δ	10	14	11	NS	NS	NS
Pulse pressure (mm. Hg)						
Baseline	39	47	61	PS	S	S
Periods X and XI	47	47	57	NS	PS	NS
Δ	−8	0	4	S	NS	S
R_s (10^3 mm. Hg) (min./cc.) (body surface m.²/1.73 m.²)						
Baseline	6.7	15.5	31.5	S	S	S
Minimum	3.6	5.7	13.6	S	S	S
Δ	3.1	9.8	17.9	S	S	S
R_s (10^3 mm. Hg) (min./cc.) (body surface m.²/1.73 m.²)						
Baseline	4.5	8.2	13.7	S	S	S
Minimum	2.0	3.4	5.2	S	S	S
Δ	2.5	4.8	8.5	S	S	S

* S = Significant (P < .01) PS = Probably Significant (P = .05 to .01) NS = Not Significant (P > .05)

The differences in per cent increments for the three age groups were not statistically significant.

Thirteen subjects exhibited temperature rises of 1° to 3° F., and two subjects developed shaking chills of 20 minutes duration. In none of these subjects was there a significant departure of the hemodynamic response from that of the rest of the group.

Filtration fraction (FF). The mean baseline FF values for the Y, M, and O age groups were 0.189, 0.206, and 0.232, respectively (Table I). The absolute fall in FF was greater in the O group (0.110) than in the M (0.083) and Y groups (0.074). At the height of the reaction the differences observed in the baseline values between age groups had disappeared (Figure 1 and Table I).

Pulse rate. The mean baseline pulse rates in the Y, M, and O groups were 67, 65, and 60, respectively (Table I). The time of onset and the absolute magnitude of pulse rate acceleration were the same for the three age groups (Figure 2 and Table I).

Blood pressures. The mean baseline systolic values for the Y, M, and O groups were 116, 124, and 136 mm. Hg, respectively (Table I). The M and O groups showed a fall in pressure while the Y group remained constant throughout the reaction (Figure 2). In periods X and XI the residual differences between the age groups are not significant.

The mean baseline diastolic pressures were approximately the same (75–77 mm. Hg) for the

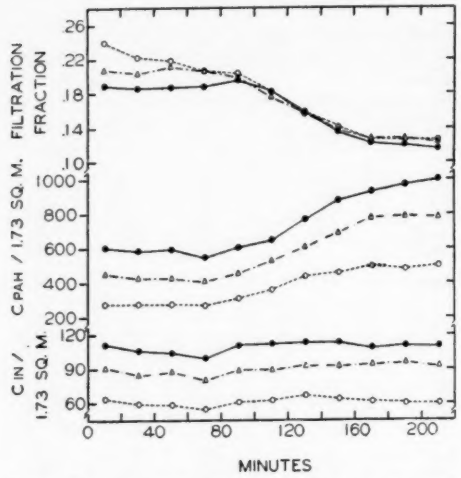

FIG. 1. CHANGES IN C_{IN}, C_{PAH}, AND FF DURING PYROGEN REACTION

Fifty million killed typhoid organisms were injected intravenously at 0 time.

○-----○ Mean value for 14 subjects in O group (70–85 yrs.)

△----△ Mean value for 20 subjects in M group (50–69 yrs.)

●——● Mean value for 20 subjects in Y group (20–49 yrs.)

different age groups. This is in part due to the setting of an upper limit of diastolic pressure by the criteria of subject selection. The average fall in diastolic pressure (Figure 2) was the same for all age groups.

A rise in pulse pressure occurred in the Y group (Figure 2 and Table I) as a result of an unchanged systolic pressure and a drop in diastolic pressure. The M group showed a commensurate fall in systolic and diastolic pressures with a resultant stable pulse pressure. The O group manifested a fall in pulse pressure due to a greater fall in systolic than in diastolic pressure. There was no demonstrable relationship between the magnitude of pulse rate or pulse pressure change, or their product, and the magnitude of the C_{PAH} response for the individuals in any age group.

Serum protein and hematocrit. Significant differences in serum protein values were observed among the three age groups. For serum protein the average values were 7.00, 6.50, and 6.10 g./100 cc. for the Y, M, and O groups, respectively. The average hematocrit values for the M and O groups were significantly lower than that for the Y group (Y = 0.44; M = 0.39; 0 = 0.39).

Renal afferent resistance (R_a). The mean baseline values for R_a were 6.7, 15.5, and 31.5 10^3 mm. Hg (min./cc.) (body surface m.²/1.73 m.²), in the Y, M, and O groups, respectively (Table I). The fall in R_a occurred in all three groups (Figure 3) and was for the O group 17.9, for the M group 9.8, and for the Y group 3.1 10^3 mm. Hg (min./cc.) (body surface m.²/1.73 m.²). Because of the mathematical relationships of the formula used to calculate R_a, the fall in these values was determined largely by the changes in mean pressure.

Renal efferent resistance (R_e). The mean baseline R_e values were higher in the O group than in the M and Y groups; the values were 13.7, 8.2, and 4.5 10^3 mm. Hg (min./cc.) (body surface m.²/1.73 m.²) respectively (Table I). At the height of the pyrogen reaction, the divergence in values was

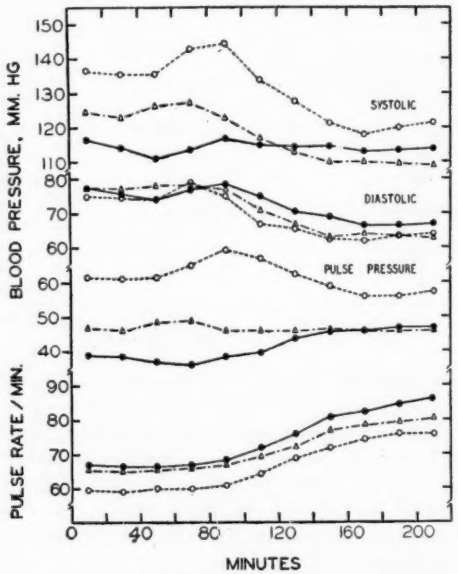

FIG. 2. CHANGES IN PULSE RATE, SYSTOLIC, DIASTOLIC, AND PULSE PRESSURE DURING PYROGEN REACTION

Fifty million killed typhoid organisms were injected intravenously at 0 time.

○-----○ Mean value for 14 subjects in O group (70–85 yrs.)

△----△ Mean value for 20 subjects in M group (50–69 yrs.)

●——● Mean value for 20 subjects in Y group (20–49 yrs.)

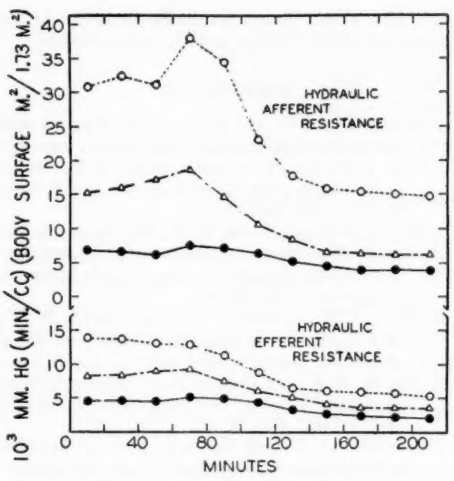

FIG. 3. CHANGES IN AFFERENT AND EFFERENT RENAL HYDRAULIC RESISTANCES DURING THE PYROGEN REACTION

Fifty million killed typhoid organisms were injected intravenously at 0 time.

O-----O Mean value for 14 subjects in O group (70–85 yrs.)

△——△ Mean value for 20 subjects in M group (50–69 yrs.)

●——● Mean value for 20 subjects in Y group (20–49 yrs.)

less (Figure 3); although, the R_e value for the O group remained significantly higher than that of the Y and M groups.

DISCUSSION

The decrease in the baseline C_{IN} and C_{PAH} in the older age groups confirms results previously reported from this laboratory (2) and are consistent with the qualitative (4, 6, 7), semi-quantitative (5), and quantitative (16) anatomic descriptions of the senile kidney. The progressively smaller absolute changes in renal plasma flow (C_{PAH}) in the older subjects following pyrogen are also consistent with a progressive decrease in the renal parenchyma of the aged. On the other hand, the time of onset of the response, and the percentage increase in renal plasma flow were similar in the different age groups. Consequently, it must be concluded that the responsiveness to pyrogen of the vascular elements remaining in the aged kidney is not qualitatively different from that of the young kidney.

Since direct evidence of structural changes in the renal vessels could not be obtained, indirect evidence was sought in clinical estimates of the degree of retinal, peripheral, and central arteriosclerosis present in the subjects tested. It is of interest that no correlation was found between the estimates of the degree of arteriosclerosis in these regions and the decrement in renal function within a given age group.

The results of this study also confirm the previously reported increase in FF with age (2). Since there is no reduction in the tubular extraction of PAH in the aged kidney (17), a reasonable explanation of the increased FF found in the O group is an increase in the efferent arteriolar resistance. The decrease in C_{IN} and C_{PAH} with age may be explained on the basis of a decrease in the number of functioning nephrons, but the observed increase in FF cannot be accounted for simply on this basis. Some change in the renal vessels beyond the point of glomerular filtration equilibrium must be postulated to account for this phenomenon. Such alteration might be present in the efferent arterioles either as structural changes, such as sclerosis, or increased vasoconstriction, or both. The fact that the FF approaches the same level in all age groups following pyrogen administration indicates that the differences are not entirely structural in nature and that the pyrogen has eliminated the inequalities of a physiologic mechanism which maintained the resting efferent arteriolar tonus at a higher level in the O than the Y group.

The calculations of renal resistances indicate an increase in both R_a and R_e in the resting state with age. During the response to pyrogen, both values diminish in subjects of all ages, so that at the height of the response the age differences are reduced but not entirely eliminated. The fall in R_a is striking in the old group as compared to that in the young. The absolute and per cent decrement observed in R_e indicates a greater degree of reversible resistance of the efferent renal arterioles in the old than in the young subjects. From the relationships between pressure and flow the presence of an initially increased R_e and the ability of the efferent arterioles of the aged kidney to dilate may be inferred.

SUMMARY

1. The renal hemodynamic response to a standard dosage of pyrogen was measured in 54 sub-

jects divided into three age groups: 20–49 years, 50–69 years, and 70–85 years.

2. The baseline C_{IN} declined progressively with advancing age. No significant change in C_{IN} occurred during the course of the pyrogen reaction in any age group.

3. The mean baseline C_{PAH} declined progressively with advancing age. During the pyrogen reaction, the proportionate increase in C_{PAH} for all age groups was approximately the same.

4. Although the baseline FF for the O group was significantly higher than the Y group, the minimum FF reached in all three groups was essentially the same.

5. The systolic blood pressure was lowest in the Y group, and did not change throughout the reaction. The systolic pressure fell significantly in the M and O groups during the reaction.

6. The diastolic blood pressure started at the same level and fell to the same degree during the reaction in all groups.

7. The baseline R_a was higher, and the fall in R_a during the reaction was greater in the older age groups than in the young group.

8. The efferent resistance (R_e) was significantly higher in each older group than in the Y group and in general paralleled the changes in FF following the administration of pyrogen. However, the minimum R_e attained by the O group remained significantly higher than the minimum R_e attained by the Y group.

CONCLUSION

It has been demonstrated that the administration of pyrogen is followed by a uniform percentage increase in renal blood flow in all age groups. Since filtration fraction fell to similar values in all age groups it is inferred that the renal arterioles are capable of dilating in the aged kidney. It is therefore concluded that the reduced renal blood flow observed in the aged is in part reversible and therefore not the result of structural changes in the renal vessels alone.

ACKNOWLEDGMENTS

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URINARY EXCRETION OF CHOLINE METABOLITES FOLLOWING CHOLINE ADMINISTRATION IN NORMALS AND PATIENTS WITH HEPATOBILIARY DISEASES[1]

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Choline has been recommended for the treatment of hepatic diseases, especially those of nutritional origin, and for the prevention of arteriosclerosis. Little is known of its fate after oral or intravenous administration. A recent Spanish report (1) that patients with liver diseases and dogs with experimental hepatic damage excrete in the urine considerably more than normal of a substance precipitated by Reinecke salt (and considered as choline by the authors) after large doses of choline given orally, suggested that the utilization of choline might be altered in hepatobiliary disease. In addition, studies with radiophosphorous (2) indicate a reduced rate of phospholipid formation in the presence of hepatocellular damage and an increased rate in biliary obstruction, suggesting that less choline might appear in the urine than normal in obstructive jaundice and more than normal in liver cell damage.

To test these hypotheses and to investigate the fate of choline after its oral administration *1*) the identity of the reineckate precipitated from the urine was determined, and *2*) the influence upon its excretion of physiologic conditions as well as hepatobiliary diseases was studied.

MATERIAL

Four normal subjects and 22 patients with various hepatobiliary diseases were given on separate occasions single doses of choline bicarbonate[3] equivalent to 2–8 grams of choline base (230–920 mg. choline N). Their diets were not controlled. Urine samples, collected at various intervals, were preserved with 25 ml. of concentrated hydrochloric acid (diluted with equal parts of water) per liter of urine.

[1] Supported by a grant from the Dr. Jerome D. Solomon Memorial Research Foundation.

[2] Solomon Foundation Fellow.

[3] We wish to thank Commercial Solvents Corporation for the generous supply of choline bicarbonate syrup.

EXPERIMENTAL

Chemical identification of the reineckate obtained from urines after choline administration and procedures applied for quantitative determination of the metabolites in the urine

Reagents used:

1) Three per cent solution of Reinecke salt, freshly prepared and filtered.
2) Aqueous saturated solution of sodium hydroxide.
3) Aqueous saturated solution of potassium carbonate.
4) Folin and Ciocalteu reagent (3), diluted with three volumes of water before use.
5) Ten per cent aqueous solution of anhydrous sodium carbonate.
6) Choline reineckate: to 40 ml. of 1 per cent choline chloride 20 ml. of the Reinecke salt solution are added. After centrifugation the precipitate is washed three times with 20 ml. portions of water. The crystals are then dried at 37° C. and stored. Immediately before use a saturated solution is freshly prepared by adding some of the choline reineckate to 0.1 normal sodium hydroxide, shaking and filtering.
7) Acetone, Norit A, Devarda's alloy, octyl alcohol, concentrated and 0.5 normal hydrochloric acid, normal and 0.1 normal sodium hydroxide.

The alkali-insoluble reineckate: The reineckate precipitated at a pH of 6.5 from urines after administration of choline (following the method of the Spanish authors [1]), was almost completely soluble in 0.1 normal sodium hydroxide. Since choline reineckate was found to be hardly soluble in this solution, the great bulk of the precipitate was not choline reineckate. On nitrogen basis, only 0.7 mg. of choline reineckate is soluble in 100 ml. of 0.1 normal sodium hydroxide while 113 mg. of urinary acid-precipitable reineckate are soluble in this solution. To determine the traces of alkali-precipitable reineckate (choline) the following procedure was used, adapted from the method of Glick (4).

Determination of choline: Saturated sodium hydroxide is added to 70 ml. of urine until a blue color to thymolphthalein appears. The precipitate is removed by filtration and to an aliquot, representing 50 ml. of urine, 25 ml. of Reinecke salt solution are added. The Reinecke precipitate formed in four hours in the refrigerator is spun

TABLE I

Recovery of choline added to urine with the method used

Diagnosis	Mg. choline N added to 100 ml.	Mg. choline N found per 100 ml.	Per cent choline N recovered
Normal	0.250	0.240	96
	0.750	0.710	93
	1.250	1.280	102
Hepatitis	0.250	0.220	88
	0.750	0.740	98
	1.250	1.360	107

down. It is washed with the saturated solution of choline reineckate in order to remove any co-precipitated noncholine reineckate. The final residue is dissolved in acetone containing 1 per cent glacial acetic acid. The solution is filtered if necessary and made to a noted volume. The transmittancy is read in a spectrophotometer or colorimeter at a wave length of 520 mμ. A standard curve is made with choline chloride using the same procedure. The results are calculated in mg. of choline N per ml. of the acetone reineckate solution. Choline added to urines from normal or hepatitis patients was recovered quantitatively (Table I). The amount demonstrated by this method represented in the studied cases 0.22 to 4.40 per cent of the total reineckate in the 24-hour urine and 0.27 to 1.04 per cent of the total amount of the choline N ingested.

The alkali-soluble reineckate: To demonstrate whether the alkali-soluble reineckate represents one of the recognized metabolites of choline such as betaine, dimethylamine or trimethylamine or its oxide, the reineckate precipitated from 10 ml. of acidified urine from normals and from patients suffering from hepatic diseases after administration of choline was dissolved in 20 ml. of 50 per cent aqueous acetone. Then 5 ml. of aqueous saturated silver sulfate solution were added (5) to remove the Reinecke ion. In the filtrate, the excess silver was removed with 5 ml. of normal hydrochloric acid and the water clear filtrate was evaporated under partial vacuum and under nitrogen at 30° C. The residue was dissolved in 10 ml. of methyl alcohol, filtered and evaporated as above. The final residue was dissolved in 20 ml. of water. An aliquot (10 ml.) was alkalinized with potassium carbonate and aerated for two hours at room temperature in the apparatus described by Sobel and associates (6) into 5 ml. of 0.5 normal hydrochloric acid. To this, 3 ml. of Reinecke salt solution were added and the crystalline precipitate obtained showed the microscopic appearance and melting point (275° C.) characteristic of trimethylamine reineckate. Mixing of the crystalline precipitate with trimethylamine reineckate did not alter the melting point. The melting point of the picrate was 216° C., identical with that of trimethylamine picrate and was also not altered by mixing with trimethylamine picrate.

To investigate whether trimethylamine oxide (TMAO) was also present in addition to trimethylamine (TMA) (the former not being aerated under the experimental conditions used), another aliquot of 10 ml. was acidified with hydrochloric acid to normality. It was then reduced with 0.5 gram of Devarda's alloy and placed in a water bath at 90° to 95° C. for 40 minutes. After centrifugation the supernatant was transferred to the aerating apparatus and enough saturated sodium hydroxide was added to redissolve the precipitate formed and then 3 ml. of saturated potassium carbonate were added. The mixture was aerated as before. In four experiments (two normal subjects and two patients with cirrhosis), the TMAO accounted for 90 to 97 per cent of the total reineckate, TMA for 5 to 10 per cent and choline for 0.56 to 0.77 per cent.

Determination of total trimethylamines (TTMA) with Reinecke salt: To 50 ml. of acid urine about 2 grams of Norit A are added and the mixture shaken and allowed

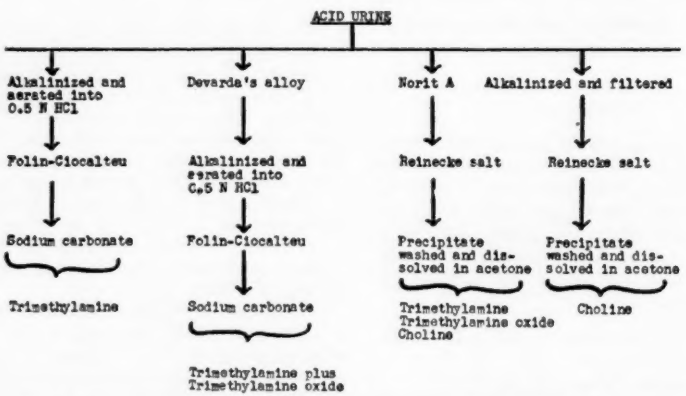

FIG. 1. ANALYTICAL SCHEME FOR THE QUANTITATIVE DETERMINATION OF CHOLINE, TRIMETHYLAMINE AND TRIMETHYLAMINE OXIDE

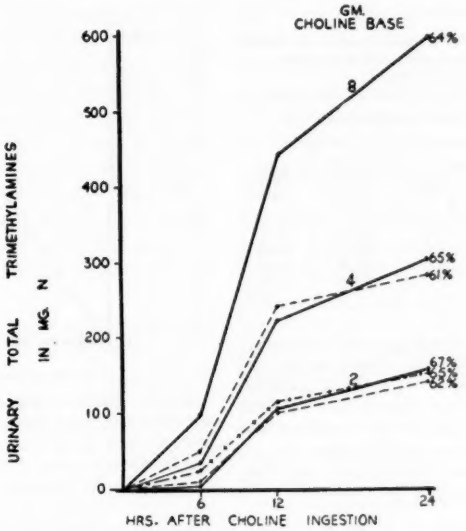

FIG. 2. URINARY EXCRETION OF TRIMETHYLAMINES BY NORMAL PERSONS AFTER ORAL ADMINISTRATION OF VARIOUS AMOUNTS OF CHOLINE

The percentage refers to the amount of trimethylamine N in relation to choline N administered. Each type of line refers to the same experimental subject.

to stand for at least 30 minutes. In some concentrated urines it is necessary to dilute the Norit filtrate. To 5 or 10 ml. of the filtrate, half a volume of the Reinecke salt solution is added. The crystalline precipitate obtained after standing at 10° C. for one hour is collected onto a fine sintered glass filter using vacuum and washed with saturated aqueous solution of "urine acid-precipitable reineckate," prepared from the same urine. The error produced by using a saturated solution of the reineckate does not exceed 20 micrograms and is, therefore, negligible. The crystals are then dissolved in acetone. The color is read in the spectrophotometer as in the determination of choline. The extinction co-efficient of the Reinecke salt of TMAO (prepared according to Dunstand and Goulding [7]), was found to be the same as that of choline, betaine and TMA chlorides when expressed as nitrogen. The very small amount of choline determined with TTMA can be ignored. The presence of betaine or other quaternary ammonium bases was excluded because the reineckate precipitable material was shown to be quantitatively removed by reduction and aeration.

Use of Folin-Ciocalteu reagent: The quantitative relation between TMA and TMAO was investigated by determining with the Folin-Ciocalteu reagent the TMA in the aerates from alkalinized urines with and without previous reduction. This reagent, otherwise used for the determination of phenols, gives a blue color with TMA (8) but not with ammonium, mono- or dimethylamine salts. Since the phenols of the urine are not aerated under the experimental conditions, the color reaction can be considered specific for TMA. This offers an alternative and simple method for the determination of TTMA even if present in small amounts. This method which has been used in this study for the determination in the stool cultures has subsequently been proven applicable for urine specimens.

Method for determination of trimethylamines with the Folin-Ciocalteu reagent: For determination of TMA, 10 ml. of urine are neutralized with saturated sodium hydroxide, followed by addition of 3 ml. of saturated potassium carbonate and 4 or 5 drops of octyl alcohol. The mixture is aerated and the TMA is collected as described before. An aliquot is neutralized with normal sodium hydroxide and made to 5 ml. with water; 2 ml. of Folin-Ciocalteu reagent and 3 ml. of the sodium carbonate solutions are added. After 15 minutes the developed color is read in a spectrophotometer at 620 mμ. A standard curve is made with pure, dried TMA hydrochloride. For determination of TTMA, 10 ml. of urine are mixed with enough concentrated hydrochloric acid to make it normal. The sample is treated with Devarda's alloy as previously described, to reduce TMAO to TMA (9). After cooling, saturated sodium hydroxide solution is added until the precipitate formed re-dissolves. Now 3 ml. of saturated po-

TABLE II

Urinary excretion of choline and trimethylamines in normal individuals within 24 hours after administration of 2 grams choline base

Choline		Total trimethylamines			
Mg. N	Per cent of choline N fed	Mg. N	Per cent of choline N fed	Per cent excreted in first 12 hours	(Choline/TMA) ×100
1.20	0.53	155.0	67.0	67.5	0.77
0.80	0.35	142.3	61.8	70.2	0.56
0.90	0.39	150.0	65.0	77.0	0.60
0.75	0.33	138.2	60.0	65.5	0.54

tassium carbonate are added and the sample is aerated as before. The aerate is conveniently diluted and treated with the Folin-Ciocalteu reagent. TMAO is calculated from the difference between the values before and after reduction. Choline and betaine are not converted into TMA and, therefore, fail to give the reaction.

The principles of the procedures applied are illustrated in Figure 1.

Variations of urinary choline and TTMA excretion under physiologic conditions

Excretion under basal conditions: In four normal subjects and seven patients with liver disease, no choline was demonstrated in 24-hour specimens of urine. In four normal subjects, 5.70 to 11.00 mg. of TTMA N and in 22 patients with liver disease, 5.80 to 14.99 mg. of TTMA N were found in 24-hour specimens of urine.

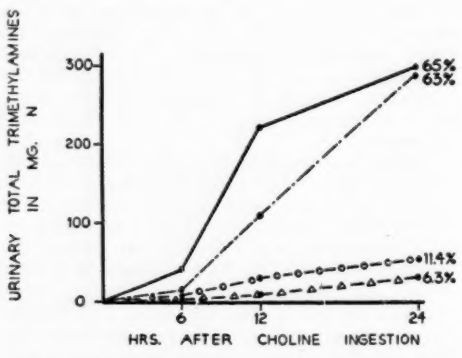

FIG. 3. URINARY EXCRETION OF TRIMETHYLAMINES AFTER ORAL ADMINISTRATION OF 4 GRAMS OF CHOLINE BASE TO A NORMAL PERSON WITHOUT SUPPLEMENT (————), AFTER FOUR DAYS TREATMENT WITH AUREOMYCIN AND SULFATHALIDINE (—○—○—), WITH SIMULTANEOUS ADMINISTRATION OF 100 GRAMS CANE SUGAR (—·—·—) AND 100 GRAMS OF STARCH (—△—△—)

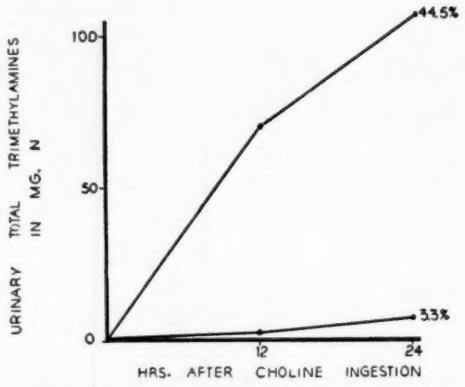

FIG. 4. URINARY EXCRETION OF TRIMETHYLAMINES BY A PATIENT WITH HEPATIC CIRRHOSIS AFTER ORAL ADMINISTRATION OF 2 GRAMS CHOLINE BASE BEFORE (UPPER LINE) AND AFTER (LOWER LINE) FOUR DAYS TREATMENT WITH AUREOMYCIN AND SULFATHALIDINE

Response to administration of choline: After administration of 8 grams of choline base to one normal individual, equal quantities of choline were excreted in any period observed. During the first 24 hours the total excretion was 1.40 mg. choline

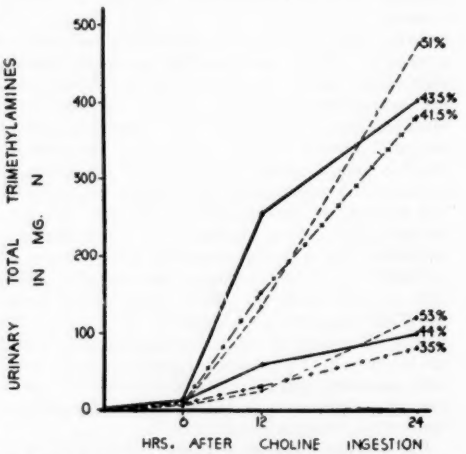

FIG. 5. URINARY EXCRETION OF TRIMETHYLAMINES BY THREE PATIENTS WITH LIVER DISEASE AFTER ADMINISTRATION OF 8 GRAMS (UPPER LINE) AND 2 GRAMS (LOWER LINE) CHOLINE BASE

Each type of line refers to the same patient.

N. Upon administration of 2 or 4 grams of choline base to four normal individuals, 0.75 to 1.60 mg. choline N were excreted in the urine. After administration of 2 to 8 grams of choline base to four normal subjects (in six experiments [Figure 2]), 60 to 67 per cent of choline N was excreted within 24 hours as TTMA of which 95 to 97 per cent was TMAO. This percentage was independent of the amount administered. Relatively little was excreted before six hours and after 12 hours, the bulk being excreted between six and 12 hours (Table II).

Effect of ingestion of other methyl compounds: Administration of 4 and 8 grams of betaine chloride or of 20 grams of dl-methionine to a normal individual did not cause urinary excretion of either choline or betaine and did not increase TTMA excretion.

Influence of intestinal bacteria upon the excretion of TTMA: Since previous investigations (10) indicated that choline is metabolized to TMA by species of the genera *Proteus, Salmonella, Eberthella* and *Shigella,* the TMA formation was determined by the aeration method in tryptose media which contained 1 per cent of choline chloride and had been inoculated with 1 drop of 1 : 1,000 saline dilution of feces. In five samples, 41 to 60 per cent of the choline present was converted to TMA in 24 hours. If 0.3 gram or less choline chloride was present in 100 ml. of tryptose medium, all choline was converted to TMA. The urinary excretion of TTMA was determined in one normal person and in two patients with cirrhosis of the liver before and after administration of 0.5 gram of aureomycin and 1 gram of sulfathalidine every four hours for four days including the day of the second choline administration. In all three instances the excretion of TTMA was greatly reduced (Figures 3 and 4 representing two of the instances). In the third one (a patient with cirrhosis) only 12-hour urine collections were available and 17.1 mg. of TTMA N instead of 78.5 mg. (without antimicrobial treatment) were excreted. Feces of the experimental subjects after treatment with aureomycin and sulfathalidine proved to be almost free of bacteria.

TABLE III

Urinary excretion of choline and trimethylamines in patients with hepatobiliary diseases within 24 hours after administration of 2 grams of choline base

Diagnosis	Choline		Total trimethylamines			
	Mg. N	Per cent of choline N fed	Mg. N	Per cent of choline N fed	Per cent excreted in first 12 hours	(Choline/TMA) ×100
Obstruct. jaundice	1.40	0.65	152.0	66.0	46.0	0.92
Obstruct. jaundice	1.30	0.56	77.1	33.6	41.5	1.70
Obstruct. jaundice	0.79	0.34	140.0	60.5	67.0	0.56
Infect. hepatitis	2.38	1.04	61.0	26.5	36.0	3.80
Infect. hepatitis	1.18	0.51	106.0	46.0	55.0	1.12
Infect. hepatitis	0.75	0.35	148.1	64.0	31.0	0.51
Infect. hepatitis	0.62	0.27	101.4	44.2	9.2	0.61
Infect. hepatitis	1.90	0.83	91.7	39.8	13.2	2.08
Chronic hepatitis	0.31	0.13	144.1	63.0	24.7	0.22
Chronic hepatitis	0.59	0.26	122.4	53.0	20.3	0.48
Cirrhosis	—	—	106.8	45.5	70.0	—
Cirrhosis	0.70	0.30	87.9	38.2	33.1	0.79
Cirrhosis	0.77	0.34	80.2	35.0	34.2	0.96
Cirrhosis with jaundice	1.10	0.48	28.6	12.4	42.0	3.85
Cirrhosis with jaundice	1.02	0.44	25.0	10.9	72.0	4.00
Cirrhosis with jaundice	—	—	80.5	35.0	38.0	—
Cirrhosis with ascites	0.65	0.28	101.0	44.0	29.5	0.64
Cirrhosis with jaundice and ascites	1.35	0.59	82.5	35.8	61.0	1.64
Cirrhosis with jaundice and ascites	1.15	0.65	26.2	11.4	61.0	4.40
Cirrhosis with jaundice and ascites	—	—	25.6	11.2	39.0	—
Cirrhosis with jaundice and ascites	1.02	0.44	82.1	35.7	39.4	1.22
Cirrhosis with jaundice and ascites	0.85	0.37	49.5	21.5	18.2	1.73

It has been shown that glucose inhibits the conversion of choline to TMA by bacteria (10). Tryptose-choline media to which 1 per cent of glucose was added, failed to reveal upon inoculation with fecal material, any TMA formation. One normal subject who received simultaneously with 4 grams of choline base, 100 grams of cane sugar, excreted in the first 12 hours half of the TMA excreted previously without sugar administration; after 24 hours the excretion did not differ from the previous test (Figure 3).

Administration of 100 grams of starch simultaneously with choline decreased the TTMA excretion in one normal person (after ingestion of 4 grams of choline base) to one-tenth, even after 24 hours (Figure 3). In a second instance (which is not graphically presented), the TTMA excretion after administration of 2 grams of choline base decreased following starch intake from 61 to 17.4 per cent of the choline N given. The urinary choline excretion did not increase.

Excretion of choline and TTMA in patients with liver diseases

In patients with liver diseases the urinary excretion of choline after administration of 2 grams of choline base was not higher than in normals. In the three patients who received 8 grams of choline base, the amount of choline excreted was higher (between 2.6 and 3.4 mg. choline N) but did not exceed one-third of 1 per cent of the total amount given.

After administration of 2 grams of choline base, two out of three patients with obstructive jaundice had a normal excretion of TTMA while the third, who died a few days later, excreted considerably less. Of seven patients with infectious hepatitis, five excreted considerably less TTMA than normal. In all 10 cases of cirrhosis investigated (two studied twice), the excretion was markedly decreased; in the two instances studied on two occasions, almost identical results were obtained. Moreover, in almost all patients with hepatic disease, the excretion was delayed in contrast to the normals. In the latter, the bulk of TTMA was excreted in the first 12 hours after the intake of choline while in the patients it was excreted between 12 and 24 hours. This delay was apparent even if the total amount excreted per 24 hours was not significantly decreased (Table III).

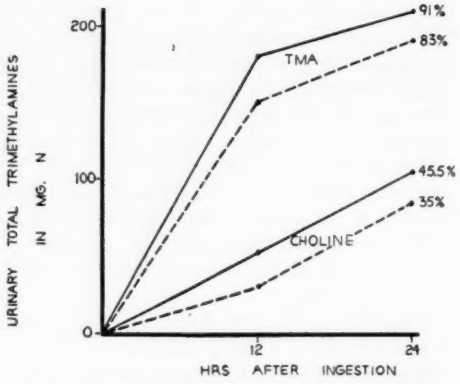

FIG. 6. URINARY EXCRETION OF TRIMETHYLAMINES AFTER ADMINISTRATION OF CHOLINE BICARBONATE AND TRIMETHYLAMINE HYDROCHLORIDE (BOTH IN AMOUNTS OF 230 MG. N) BY TWO PATIENTS WITH HEPATIC CIRRHOSIS

Each type of line refers to the same patient.

In three patients with liver disease, the percentage of TTMA N excreted in relation to the amount of choline N ingested was not significantly changed when 8 grams instead of 2 grams of choline base were given (Figure 5). Two patients with liver disease who previously had delayed and reduced TTMA excretion after choline administration and who ingested TMA hydrochloride in amounts equivalent to 230 mg. N excreted it rapidly and almost completely (Figure 6).

DISCUSSION

In contradiction to the previously cited report on urinary choline excretion (1), this substance is excreted in the urine in insignificant amounts by normal subjects and patients with liver diseases with or without administration of large doses of choline. With the method used, the excretion rarely exceeded one-third of 1 per cent of the administered dose. Moreover, reasonable doubt exists as to whether the very small precipitate represents choline and the figures given are maximum rather than actual amounts. The bulk of the reineckate obtained in the urine after choline administration (independent of the quantity administered) was proven to be trimethylamine (TMA). The greatest part of TMA was in the oxide form. This is in keeping with previous reports that in

rats (11) and in one human (12) after choline administration, TMA and TMAO appeared in the urine.

This study indicates that, as measured by two different methods, two-thirds of the choline administered appears in the urine as TMA or its oxide. The absolute amount is astonishingly well duplicated in the same person. Since many bacteria, especially those of the *Proteus* and *Shigella* groups, are known to transform choline into TMA (10), the possibility of such a process in the intestinal tract had to be considered. It was confirmed by the observation that *in vitro* feces transform choline, to a large degree, to TMA and that suppression of the intestinal flora by antimicrobial agents greatly inhibits the urinary total trimethylamines (TTMA) excretion without influence upon the choline excretion. The conclusion is justified that two-thirds of choline administered as dietary supplement, independent of the dose given, is transformed in the intestinal tract into TMA. Information as to the lipotropic activity of the latter is not available. Neither betaine nor methionine is transformed or excreted as TTMA. The fate of the remaining one-third of choline in the body is unknown.

The concept of an extensive bacterial transformation of choline into TMA appears strange in view of the fact that choline absorption should take place in the upper part of the small intestine while significant bacterial activity starts in the large intestine. Further investigations should clarify the extent of absorption of choline as such in different species.

Glucose, which is known to suppress TMA production from choline by bacteria, inhibits *in vitro* production of TMA by feces. Therefore, the suppression of intestinal TMA formation (as measured by urinary TTMA excretion) by administration of choline together with carbohydrates was attempted. Cane sugar, which is rapidly absorbed from the intestine, delayed the TTMA excretion (and probably the bacterial transformation) without reducing the total excretion in 24 hours. In contrast, starch given with choline for the entire 24-hour period considerably depressed the bacterial transformation of choline if the urinary excretion of TTMA is a measurement.

Three possible explanations have to be considered for the observed reduction or delay of the urinary TTMA excretion after choline intake found in hepatic diseases:

(*a*) Impaired absorption of TMA in the presence of liver damage. The almost complete urinary excretion of ingested TMA even in patients with liver disease speaks against this assumption.

(*b*) Faulty urinary excretion of TTMA. The complete excretion of fed TMA in liver diseases contradicts this possibility also. The bulk of TMA is excreted as its oxide. The possibility, therefore, has to be considered that the oxidized form is more readily eliminated by the kidneys; if the liver performs this oxidation, in liver diseases this function could be at fault. *In vitro* studies (13) have indicated that liver tissue reduces TMAO to TMA; therefore, the fact that the ratio between urinary TMA and TMAO is not significantly altered in liver diseases contradicts this assumption.

(*c*) Faulty bacterial action in liver diseases. Possibly the bacterial flora of the intestine is altered in patients with hepatobiliary diseases in a quantitative and/or qualitative manner which might explain the reduced formation of TMA in these conditions.

The data so far available are too scant to permit conclusions about the therapeutic value of supplementing choline therapy with antimicrobial agents or carbohydrates or about the causes of choline deficiency in the human. The utilization of orally administered choline as measured by phospholipid turnover with P^{32} (14) cannot, at present, be correlated with the urinary excretion of TTMA since the latter does not reflect the fate of absorbed choline in the body. For this purpose, studies on the urinary excretion of intravenously administered choline, which are at present under way, appear more promising.

SUMMARY

1. A procedure was adopted for the determination of choline in the urine using its precipitation by Reinecke salt, and procedures were developed for the determination of trimethylamine and trimethylamine oxide in the urine, based on either Reinecke salt precipitation or on the Folin-Ciocalteu reaction. These procedures were applied to the urines of four normal persons and 22 patients with hepatobiliary diseases before and after oral administration of 2 to 8 grams of choline base.

2. Choline was not found in the urines of normal persons nor patients with hepatobiliary diseases under basal conditions. After oral administration of choline, very small amounts, if any, of choline were excreted, as a rule not exceeding 0.3 per cent of the amount of choline administered.

3. Under basal conditions, small amounts of trimethylamines were found in the urine. Within 24 hours about two-thirds of the choline N ingested was excreted in normal individuals as trimethylamine and its oxide.

4. Incubated stool dilutions transformed choline to trimethylamine. Inhibition of the intestinal flora by aureomycin and sulfathalidine greatly depressed the urinary trimethylamine excretion. This suggests that the urinary trimethylamines result from bacterial transformation of choline in the intestine. The greater part of therapeutically administered choline is, therefore, changed in the liver into a product without known lipotropic activity.

5. Administration of cane sugar with choline delayed urinary trimethylamine excretion, while intake of starch depressed it.

6. In liver diseases the urinary trimethylamine elimination after choline administration is delayed or decreased. Ingested trimethylamine in these patients is readily excreted.

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THE NEPHROTIC SYNDROME IN CHILDREN. AN INTERPRETATION OF ITS CLINICAL, BIOCHEMICAL, AND RENAL HEMODYNAMIC FEATURES AS VARIATIONS OF A SINGLE TYPE OF NEPHRON DISEASE [1, 2]

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It is the purpose of this report to describe and interpret the varied renal hemodynamic patterns and associated clinical and biochemical features observed in 29 children with the nephrotic syndrome studied between January, 1949, and June, 1950.

Cases of the "nephrotic syndrome" present certain uniform and consistent clinical features (1), but their separation into two groups, "nephrosis" and the "nephrotic stage of nephritis," has been rather arbitrary and frequently difficult. Normal or supernormal renal function (2) on the one hand, and hematuria, hypertension, and azotemia (3–6) on the other, have been the classical criteria for this distinction. However, review of the clinical course of patients classified as "nephrosis" with subsequent complete remission, has shown that moderate hematuria, hypertension and azotemia were common transiently observed phenomena (7–10). Moreover, serial renal function studies have demonstrated varying degrees of functional impairment in patients clinically classified as instances of either nephrosis (11, 12) or the nephrotic stage of glomerulonephritis (13–15).

This study was undertaken in order to determine:

1) If measurements of renal function would assist in the early differentiation of benign nephrosis from progressive nephritis,

2) if such a differentiation were valid physiologically, and

3) if studies of renal function would help to elucidate the pathogenesis of the nephrotic syndrome. A preliminary report has been published (16).

Lacking the biopsy material which would permit morphologic description of the diseased organ, the results of our physiologic studies seem to corroborate an earlier suggestion, based on pathologic studies, that both "degenerative" lipoid nephrosis and "inflammatory" glomerulonephritis are characterized by anatomical lesions and disturbances in function which are identical in kind, but different in degree (17). The data of this report indicate that the glomerulus is a primary site of the disease process, but the syndrome is frequently characterized by reversible metabolic and hemodynamic rather than solely by irreversible anatomic damage of the nephron.

CLINICAL MATERIAL AND PROCEDURES

General plan of study

Estimations of glomerular filtration rates (GFR), renal plasma flow (RPF), and maximal tubular excretory capacity (Tm_{PAH}), were made in 29 nephrotic children, with more than one series of simultaneous biochemical and renal functional studies in 17 of these patients. In all, 53 estimations of renal function and biochemical status were made and correlated with clinical courses. Of the 29 patients, only two were over 6.5 years of age at the time of first admission. Three patients were over 6.5 years of age at the time initial renal function studies

[1] These studies were supported by grants from Mead Johnson & Co. and from the National Institutes of Health, U. S. Public Health Service.

[2] Presented in part before the Society of Pediatric Research, French Lick, Ind., May 1950, and the Sixth International Congress of Pediatrics, Zurich, Switzerland, July 1950.

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TABLE I

Clinical status of all nephrotic patients observed during interval January, 1949–June, 1950

Nephrotic patients	No. of patients	Males	Clinical status*			
			Remission	Good	Poor	Dead
This report	29	11	10†	6	3	10‡
Remainder clinic group	61	45	20	22	9	10
Total	90	56	30	28	12	20
Per cent	100	62	33	31	14	22

* Remission = No edema, normal diet, full activity, minimal to absent proteinuria for at least two months.

Good = Edema, proteinuria, hypoproteinuria, hyperlipemia, no anemia, acidosis, azotemia. Urine specific gravity 1.018 or more.

Poor = Nephrotic syndrome with anemia, acidosis, azotemia, urine specific gravity "fixed" 1.010.

† Three patients have relapsed since this report was submitted for publication.

‡ Two patients died shortly after this report was prepared for publication.

were done. All other patients were less than five years of age. Omitting two extremes of age, the mean age of the group was 3.3 years and median age 3.0 years. Comments on the physiological disturbances of electrolytes and body fluids will be the subject of another report.

Appraisal of the factors which determined the selection of the particular patients for study from our clinic group was extremely difficult. Patients usually appeared in the clinic soon after manifestations of the disease were noted. The observed group of patients, therefore, was not unduly weighted by the usual preponderance of severe or advanced cases commonly noted in hospital samples. An attempt was made to select for study children with nephrosis and both with and without hematuria and/or azotemia, and with active disease of varying duration.

The sex distribution and clinical status of the patients studied is related to that of the total group of 90 nephrotic children observed in this clinic during the same time interval (Table I). The inclusion of fewer males in the study sample may be attributed to reluctance to catheterize male children with marked edema of the genitalia.

Blood samples

Serum or plasma concentrations of the test substances employed were, in the majority of instances, measured in "arterial" blood obtained from capillary puncture. An inlying venous needle with fitted stylette was frequently used in larger children for sampling. Approximately 0.5 ml. whole blood was collected in 10 cm. long, 3 mm. bore, chemically clean capillary tubes—filled by direct capillarity from finger puncture (18). A drop of dilute heparin was used to wet the bore when plasma was desired; however, a considerably better yield of serum was usually obtained from the same aliquot of non-heparinized blood. The capillary tube, when filled, was capped with flanged flexible rubber caps.[4] Twenty to 40 seconds were usually required to fill a tube. After centrifugation for 30 minutes at 2,500 rpm, the tube was scratched 1 mm. above the level of packed cells, the cap at the serum end of the tube removed, and the serum-containing portion of the tube carefully broken off and recapped. The heparinized blood hematocrit was read directly by alignment of the tube against a millimeter rule. Approximately 0.2–0.3 ml. serum was thus available per tube. Two or three microtubes obtained per period sufficed for duplicate determinations. Where simultaneous thiosulfate, inulin, and PAH clearances were done, four or five tubes were obtained per period. The relatively small yield of serum, however, necessitated micromodification of usual procedures.

Renal function studies

Patients were hospitalized and confined to bed for 18 to 24 hours prior to study. Fluids were allowed *ad lib.* No food was offered during the eight hour period preceding the test. All clearance studies were begun at approximately 8:30 a.m. Using a dose based on age, premedication with morphine sulfate and atropine sulfate was employed in about two-thirds of the studies in order to reduce apprehension in uncomfortable, edematous children. In general, commonly accepted techniques with few minor modifications were employed. A single injection of thiosulfate and constant infusion of inulin and PAH were used. Urine was collected through an indwelling, multi-eyed 8–14F catheter. The bladder was washed at the end of each period with sterile distilled water and with air. Urine volumes, including wash, were recorded to the nearest 0.1 ml. Thirty minutes were allowed for equilibration after the priming injection. Consecutive 15 minute collections of urine were then made for six to ten periods. In the majority of instances, blood samples were obtained at approximately 30, 60, 90, 120, 150, and 180 minutes and midpoint values of each period determined by interpolation. It was not possible to correct for urine delay time. A control venous blood sample was obtained under oil for blank determination and analysis of various serum chemical components. All sera and urine aliquots for thiosulfate and inulin analysis were precipitated within two to four hours. Analyses were completed within 24 hours.

METHODS

Glomerular filtration rate (GFR) was determined using a single injection of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)

[4] Obtained from the West Company, 1117 Shakamaxon St., Philadelphia, Pa.

(19) in the initial 19 observations. In the majority of instances, simultaneous inulin and thiosulfate clearances were measured. Approximately 30 mM. (8 gm.) $Na_2S_2O_3 \cdot 5H_2O$ per square meter of surface area were administered as a freshly prepared 10–17% solution. Unpleasant reactions to relatively rapid infusions (6–10 ml./min.) of this solution were rare. Concentrations of thiosulfate in serum and urine were determined in duplicate, with micromodification for serum, according to the standard periodate titrometric method. Semilogarithmic plot of urinary thiosulfate excretion and of serum concentrations against time usually followed decremental straight lines of varying slope. Initial serum concentrations approximating 40–60 mg./100 ml. were usually obtained.

The inulin clearance determined by a constant infusion technique was measured simultaneously with thiosulfate and PAH clearances in the majority of instances.[5] Serum concentrations of inulin usually approximated 20–80 mg./100 ml., and of PAH, 1.5–4.0 mg./100 ml. Inulin in serum and urine was determined by method of Roe, Epstein, and Goldstein (21) with minor modification. The standard acid $CdSO_4$ protein precipitating solution (22) was found to give as satisfactory recoveries as the Somogyi reagents, and therefore this deproteinizing agent was used. For precipitation, serum in the microtubes was transferred by capillarity into a calibrated 0.2 ml. (normax grade) $\overline{\text{C}}$ pipette and delivered with three washings into 2.0 ml. distilled water contained in a 3–4 ml., 10 mm. bore, precipitin tube. The standard $CdSO_4$ precipitation was completed using one-tenth quantities of reagents. After standing 10 minutes, the precipitate was compacted by centrifugation for 20 minutes at 2,000 rpm. Duplicate tubes were prepared for each specimen and afforded sufficient supernatant for duplicate determinations of PAH and inulin. Optical density of color for inulin and PAH was determined in a Coleman Junior spectrophotometer. Maximum absorption of the resorcinol-inulin complex occurred at 510 mμ. Standard recoveries were determined with each series of analyses and an average constant in terms of optical density was derived. The constant, rather than a standard transmission curve, was used for calculation. Little variation in constants was observed in more than 70 multiple recovery determinations.

Constant infusions of PAH following a priming dose were used to measure renal plasma flow (RPF) and maximal tubular excretory capacity (Tm) (23). PAH was diluted in 5% glucose for clearance and in distilled H_2O for Tm determinations. Priming doses were diluted in distilled H_2O. The observed PAH clearances may be low because of the unrecognized reaction of PAH with glucose (24). In the initial renal function studies, glomerular filtration rates were determined with thiosulfate immediately prior to RPF and subsequent Tm periods. Thereafter, simultaneous inulin and PAH clearance and saturation studies were carried out. Concentrations of PAH in serum and urine were determined by micromodification of a standard method (22). At high serum concentrations, 1.0 ml. aliquots of the supernatant solution obtained after precipitation (*vide supra*) were diluted 20 times with distilled water and color was developed in 10 ml. aliquots by standard macro technique. Color was read at 540 mμ in the Coleman Junior spectrophotometer. Standard recoveries and determination of recovery constants were carried out with each series of analyses. Serum blank was determined by difference. Urine blanks were found to be negligible at the dilutions used (1 : 2,500–10,000).

Impairment of the renal tubules may decrease the extraction ratio and may invalidate the use of uncorrected clearances for the measurement of renal plasma flow. Renal PAH extraction data are not yet available in infants and children. Cargill has reported values for renal PAH extraction of normal adult patients and of nine glomerulonephritics with varying levels of observed PAH clearance (25*a*). An exponential extraction curve has been constructed from these data and used to "correct" observed RPF for approximate extraction in order to afford an estimate of "corrected" renal plasma flow (RPF_c). An assumption of this type has obvious limitations, but occasionally seemed to yield a more reasonable range of magnitude for renal plasma flow.

Estimation of surface area

The estimation of surface area in the persistently edematous child presents an obvious problem, and ideal weight in relation to height and age is unreliable in children whose disease is of sufficiently long duration to impair linear growth and induce chronic malnutrition with wasting. A reasonable approximation of "non-edematous weight" usually was derived by comparing the observed volume of distribution (V_D) of thiosulfate in the edematous patient to a presumed "normal" V_D estimated from thiocyanate space regression equations for height (25*b*). SCN and S_2O_3 may occupy somewhat different apparent distributions in view of the protein binding of SCN (26);

[5] A comparison of 118 simultaneous thiosulfate and inulin clearance periods indicated fairly good agreement at all clearance levels, although the quantitative significance of values obtained at apparently low levels of filtration is questionable. Occasional widely discrepant values were noted. Thiosulfate clearances were usually greater than those of inulin. The average clearance ratio $\frac{\text{thiosulfate}}{\text{inulin}}$ for all periods was 1.1. Greater discrepancies were observed with the highest clearance values. The correlation coefficient of thiosulfate versus inulin clearance was 0.88 ± 0.09. The mean discrepancy between all values for simultaneous thiosulfate versus inulin clearance was 5.5 ± 3.9 ml./min. The ratio, $\frac{\text{C thiosulfate,}}{\text{C inulin}}$ was apparently independent of serum concentration of thiosulfate, $r = 0.36$, in contrast to observations in pregnant women (20).

neither accurately measures extracellular fluid volume.[6] Occasionally such estimation of surface area (SA) was obviously inadequate. Post-diuresis weight then was used to correct previous measurements of renal function to unit values. Using the derived value for "non-edematous weight," SA was estimated either by calculation or from nomograms constructed from the standard DuBois relationship: $SA = W^{0.425} \times H^{0.725} \times 71.84$.

Other measurements

Serum proteins were determined by micro-Kjeldahl analysis. Serum NPN (27), gasometric CO_2 content (28), pH (29), and cholesterol (30), were determined by standard methods. Plasma volume determinations,[7] using Evans blue (T-1824) and a modification of an acetone extraction technique (31), were carried out in all patients immediately prior to clearance studies.

RESULTS

I. Clinical observations

A summary of the clinical features of patients studied by clearance techniques is given in Table II.

[6] The use of V_D thiosulfate for the estimation of "non-edematous weight" is indicated by the following example:

Patient CB, Male—Age 1–11/12
Edematous—Before diuresis

Height = 90.8 cm.		
Initial volume distribution (V_D) $Na_2S_2O_3$		= 8.26 L.
Predicted normal V_D for height*		= 3.32 L.
Estimated volume edema (predicted decrease V_D)		= 4.94 L.
Estimated weight edema = 4.94 × 1.02		= 5.04 Kg.
Actual weight	17.70 Kg.	
Edema	5.04	
Estimated non-edematous weight for predicted change in V_D	12.66 Kg.	

* Where V_D (NaScN) = −6.14 + 0.1042 H for SA = 0.5 − 1.2 M^2 (25*b*).

Non-edematous—After diuresis

Height = 91.5 cm.		
Final volume distribution (V_D) $Na_2S_2O_3$		= 3.23 L.
Observed decrease (V_D), 8.26 − 3.23		= 5.03 L.
Estimated weight change from observed change in V_D = 5.03 × 1.02		= 5.13 Kg.
Predicted weight from actual V_D, 17.70 − 5.13	= 12.57 Kg.	
Observed non-edematous weight	= 11.88 Kg.	
Error of prediction	= 0.69 Kg.	6%
Estimated ideal weight from height	= 13.50 Kg.	
	11.88	
Error of prediction	= 1.62 Kg.	14%

[7] Detailed data will be the subject of another report.

The onset of symptoms was estimated from history. Since many of the children who developed the disease were quite young, observations of growth and physical status, occasionally including blood pressure and urine examination, had often been made by the family physician prior to onset of the nephrotic syndrome. When available, such information confirmed the absence of antecedent renal disease. Since renal disease of this type in children is usually associated with marked loss of appetite, lassitude, oliguria, and personality change or irritability before edema is more than suspected, parents were apt to consult their physician with the advent of definite edema. In no instance was gross hematuria, elevated blood pressure, or cardiac failure observed by the first physician to see the patient in the early phases of the disease. Upper respiratory or other infections preceded the onset of the nephrotic syndrome in only seven of 29 patients.

All patients with the nephrotic syndrome at the time of initial observation in this clinic presented the rapid or insidious onset of edema as a chief complaint. Oliguria, marked proteinuria, absence of gross or marked microscopic hematuria, hypoproteinemia (micro-Kjeldahl), and elevation of serum cholesterol were constant features. Cardiac enlargement was not observed but variable values for blood pressure and NPN were obtained in all phases of the disease (Table II). Spontaneous diuresis, frequently observed in the first weeks or months of the disease, was usually associated with temporary improvement in the values for urine and blood constituents.

Hematuria, azotemia and anemia

The clinical data indicated that hypertension, microscopic hematuria, and/or slight to moderate azotemia during the early months of the disease often were unreliable indices of the functional state of the kidney or subsequent course of the disease. However, essentially hyperbolic correlation was found between elevation of the NPN and diminution of GFR (Figure 1) as previously noted (32–34). Similarly, the occurrence and extent of acidosis, as indicated by serum CO_2 content and pH, was found to correlate roughly with reduction of GFR (35, 36) (Figure 2). The simultaneous variation of pH, CO_2 content and non-CO_2 serum buffers in relation to GFR was expressed according

TABLE II

Clinical and laboratory data of children with the nephrotic syndrome

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
				Laboratory data													Clinical data		
				Urine†			Blood chemistry								Infection				
Case number	History number	Patient Age on first admission	Duration of disease in months*	Protein	RBC	Casts	NPN mg.%	CO_2 content mEq./L.	pH	Serum proteins gm.%	Cholesterol mg.%	Hgb gm.%	Edema‡	Blood pressure	Before onset	After onset	Special therapy	Diuresis	Status Months after onset
1	354053	S. J. 4-5/12 ♀	0.4 1.7 3.1 4.0 5.5	0.9 1+ 1.2 1.5 0	0.2 1+ 0.2 0 0.4	0 1+ 0.3 0.4 0	51–25 27.6 35.4 26.8 25.1	19 24.9 21.5 27.6 24.0	7.4 7.4 7.3 7.4 7.4	3.8 5.5 3.5 4.7 5.8	590 750 395 260 140	15.5 13.5 12.4 15.2 11.7	1+ 1+ 1+ 0 0	110/70 102/60 120/70 104/70 112/80	None	Atypical pneumonia 1.5 mo.	ACTH 4 mo.; prophylactic antibiotics	Spontaneous post pneumonia; post ACTH	Temp. remission 6 mo.
2	355028	C. P. 1-4/12 ♀	0.6 4.5 5.2	3+ 3+ ±	4–10 2–4 Rare	4 4	63.6 33.1 25.7	20.6 20.0 30.5	7.2 7.2 7.4	4.0 4.7 4.9	680 615	9.5 8.1 9.2	1+ 2+ 0	110/60 100/60 114/64	Diarrhea etiology? 0.5 mo.	None	ACTH 5 mo.; antibiotics	Initial spontaneous; post ACTH	Remission 6 mo.
3	338957	A. G. 1-3/12 ♀	1.0 14.0	3+ 2+	2–4 2–4	4 4	60.0 39.3	18.3 11.3	7.2 7.2	3.6 2.9	485	15.0 12.8	4+ 1+	116/70 120/80	None	None	Multiple paracenteses and transfusions	Early in course	Died. Reported uremia and cardiac failure 16 mo.§
4	359815	L. A. 2-3/12 ♀	1.0	3+	2–4	0–2	22.4	25.8	7.4	4.2	570	12.0	1+	110/70	None	URI	Prophylactic antibiotics	Spontaneous 2 wks. post onset; 3–4 times since	Intermittent edema, proteinuria 3 mo.
5	339231	R. S. 6-6/12 ♂	1.0 14.0	3+ 3+	0–10 2–4	0–4 0–1	61.4 55.9	22.3 19.0	7.4 7.3	3.7 3.4		13.6 8.0	4+ 1+	130/98 120/70	URI 0.5 mo.	None	Thoracenteses; paracenteses	None	Died.¶ Uremia 17 mo.
6	339226	R. M. 2-7/12 ♂	1.5	3+	10	2–4	47.4			4.2		16.0	2+	130/100	URI 0.5 mo.	*B. coli* 5 mo.; alpha strep. septicemia 7 mo.	Thoracentesis; antibiotics	Post *B. coli* infection	Died.** Alpha streptococcus septicemia 7 mo.
7	339937	B. D. 1-8/12 ♀	2.0	3–4+	0–10	0–2	105.0	24.3	7.3	3.7	445	13.4	3+	125/70	Vaccinia 0.4 mo.	None	None	None	Unknown 20 mo.
8	353096	G. B. 1-10/12 ♂	2.0 2.8 8.8	0.8 0.6 4+	5.0 1.4 6–8	1.0 0 1–2	48.3 22.1 52.9	21.7 23.3 20.4	7.4 7.3 7.2	4.1 5.5 3.2	800 700	13.4 10.2 8.8	4+ ± 4+	118/88 98/82 120/90	None	Cellulitis 5 mo.	ACTH 2.5 and 11 mo.	Post 1st ACTH only	Early renal failure 9 mo.
9	356191	H. M. 3-4/12 ♂	2.5 5.0	0.5 3+	0.4 9	0 1–2	35.4 36.4	27.5 27.8	7.4 7.2	4.3 4.0	400 615	12.2 13.5	1+ 3+	108/60 120/60	URI 0.3 mo.	"Tonsillitis" 1 mo.	ACTH 5 mo.; paracenteses; antibiotics	Initial spontaneous 2 wks.; none since	Active disease 6 mo.
10	339952	J. M. 4-8/12 ♀	4.0 18.0	3–4+ 3–4+	0–2 0–2	0–1 0–1	30.0 31.3	23.8 16.7	7.5 7.3	4.8 3.6	444 470	14.0 9.7	2+ 1–2+	106/90 94/70	None	Recurrent cellulitis and bronchitis	Transfusions	Spontaneous 5 and 18 mo.	No edema, uremia 21 mo.
11	338069	F. K. 2-3/12 ♂	4.0 16.0	3.5 4.6	0.2 39.6	0 1.8	33.1 91.6	25.3 13.4	7.4 7.1	3.5 3.8		14.6 5.3	3+ 1+	90/70 92/78	None	None	Human serum albumin	Spontaneous 1 mo.; again with uremia 14 mo.	Died. Uremia 17 mo.§
12	359828	J. Ma. 3-0/12 ♂	4.0 4.7	4+ 0.1	5.1 1.6	19.7 0.1	60.6 60.6	21.9 27.7	7.3 7.4	3.8 4.3	570 470	11.7 10.5	3+ ±	106/68 108/64	URI 0.3 mo.	None	ACTH 4 mo.	Spontaneous 1 wk. and post ACTH	Remission 6 mo.

TABLE II—*Continued*

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
				Laboratory data													Clinical data		
				Urine†			Blood chemistry								Infection				
Case number	History number	Patient Age on first admission	Duration of disease in months*	Protein	RBC	Casts	NPN mg.%	CO_2 content mEq./L.	pH	Serum proteins gm.%	Cholesterol mg.%	Hgb gm.%	Edema‡	Blood pressure	Before onset	After onset	Special therapy	Diuresis	Status Months after onset
13	337285	J. F. 3-11/12 ♀	4.0 17.0	3+ 4+	0–2 4–10	0–1 4–10	39.2 39.7	25.5 21.2	7.4 7.3	3.2 4.4	730	12.0 12.6	2+ 0	100/60 86/48	None	Probable peritonitis 2 times in first 18 mo.	None	Spontaneous 17 mo.	No edema, uremia 35 mo.
14	353044	G. C. 2-1/12 ♂	5.0	0.9	1.1	0.7	33.1	24.9	7.4	3.1		13.8	2+	90/60	Severe sunburn 0.3 mo.	None	None	Spontaneous 1 and 8 mo. with remission	Remission 12 mo.
15	342134	R. G. 3-1/12 ♂	6.0 7.5	4+ 3+	0–2 2–4	0–1 4–10	25.9	24.0 26.4	7.4 7.2	3.2 3.6		12.3 10.5	3+ 2+	116/78 112/70	None	Cellulitis; pneumonia; pneumococcal septicemia; terminal staph. albus septicemia	Human serum albumin; measles 7 mo.; thoracenteses, paracenteses; ACTH 15 mo.	None	Died.†† Cardiac failure 17 mo.
16	338933	P. N. 2-8/12 ♀	6.0	4.5	2.3	0.5	70–23	24.4	7.4	3.4	478	13.8	3+	130/96	None	Measles	Measles 3 mo.	Following measles and 2 times since	No edema,¶ proteinuria 25 mo.
17	350433	R. B. 1-11/12 ♂	7.0 11.0 12.0	2.2 1.5 1.5	4.8 55.0 0.7	2.0 1.2 0.1	24.7 45.8 29.1	20.2 14.4 24.8	7.4 7.2 7.4	5.0 3.2 4.2	460 304	10.8 8.4 10.2	1+ 4+ ±	110/70 125/75 98/52	None	None	ACTH 6 and 11 mo.	Post ACTH	Temporary remission 14 mo.
18	328927	E. D. 2-0/12 ♀	7.0 7.5 22.0	3+ 2+ 3+	10+ 4–5 0–3	0–1 0–1	67.7 69.7 98.0	24.0 24.0 12.9	7.3 7.3 7.2	2.9 3.2 3.2	730 800	12.0 10.2 6.2	4+ ± 2+	110/70 100/60 110/80	None	None	Human serum albumin; frequent paracenteses and transfusions	Post paracentesis	Died.‡‡ Uremia 30 mo.
19	346666	H. S. 3-0/12 ♂	8.0 12.7	3+ 0.4	0–10 0	4–10 2.0	52.8 33.1	21.2 21.8	7.3 7.2	3.8 3.7	412	11.0 10.8	2+ 3+	100/70 110/80	URI 0.5 mo.	Paracolon septicemia 11 mo.; cellulitis 12 mo.	Paracenteses, thoracenteses; ACTH 13 mo.	Post paracenteses; post ACTH	Remission 14 mo.
20	342976	D. G. 7-8/12 ♀	9.0	2.5	5.3	6.9	45.8					14.2	3+	106/72	None	Peritonitis; cellulitis	None	Following cellulitis	Active disease 24 mo.
21	343049	L. C. 3-1/12 ♀	15.0	3+	2–2	0–1	26.8	24.3	7.4	5.3		14.7	0	98/58	URI 0.3 mo.	Bronchitis 5 mo.; peritonitis 9 mo.	Prophylactic antibiotics	Spontaneous 10 mo., with remission	Remission 20 mo.
22	342905	H. O. 14-6/12 ♀	15.0 26.0	6.8 4+	63.4 4–10	3.9 0–1	43.7 26.9	28.0 25.2	7.4 7.3	3.1 2.8	650	17.5 10.4	4+ 1+	158/116 200/128	None‖	None	Human serum albumin	None	Active disease 30 mo.
23	329846	W. L. 2-10/12 ♂	15.5	3+	10+	0–1	116.8	9.0	7.2	3.9		9.5	1+	110/80	URI	Mumps 0.5 mo. Pneumococcal peritonitis 8, 10, 13 mo.; pneumonia	Human serum albumin	Post infection 8 mo.	Died.§§ Uremia 16 mo.
24	344988	E. G. 4-2/12 ♀	17.0 30.0	3+ ±	4–10 0–2	0–1 0	37.3 23.4	21.2 29.5	7.1 7.4	3.0 5.4	756 540	10.4	4+ 0	124/80 110/65	Measles 1 mo.	Cellulitis; bronchitis	Human serum albumin	Spontaneous 2 yrs.	Remission 34 mo.

TABLE II—*Continued*

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
				Laboratory data													Clinical data		
				Urine†			Blood chemistry								Infection				
Case number	History number	Patient Age on first admission	Duration of disease in months*	Protein	RBC	Casts	NPN mg.%	CO_2 content mEq./L.	pH	Serum proteins gm.%	Cholesterol mg.%	Hgb gm.%	Edema‡	Blood pressure	Before onset	After onset	Special therapy	Diuresis	Status Months after onset
25	353073	S. S. 4-9/12 ♀	18.5 19.0	0.6 2.4	0.8 0.6	2.2 0.7	38.8 44.6	22.0 19.8	7.3 7.2	3.2 3.4	475 475	10.2	2+ 3+	106/70 130/90	None	Frequent bronchitis; probable peritonitis	Paracenteses; ACTH 17 mo. prophylactic antibiotics	Post ACTH and paracentesis with remission	Remission 24 mo.
26	325742	B. S. 3-10/12 ♀	20.0	4+	0–2	4–10	152.0	15.2	7.3	4.7	*	9.0	2+	118/100	None	Measles 5 mo.	Frequent paracenteses	Minimal post measles	Died 33 mo.§
27	330624	L. N. 2-7/12 ♀	22.0	3+	0–2	4–10	65.0	9.2	7.3	3.2		8.5	1+	90/50	None	Peritonitis; cellulitis	Human serum albumin; paracenteses	Post infection 9 mo.; spontaneous 22 mo.	Died. Uremia 36 mo.§
28	341502	E. M. 4-6/12 ♀	35.0	3+	0–10	0–1	72.8	11.9	7.3	3.8	365	8.4	4+	120/88	None	Uncertain	Frequent paracenteses	None	Died. Uremia, cardiac failure 37 mo.§
29	269313	A. O. 2-7/12 ♀	64.0	3+	0–2	0	30.8	27.8	7.5	4.8		14.8	0	104/70	URI	Measles 13 mo.; pneumococcal septicemia 26 mo.	Measles; human serum albumin	Post measles; with albumin 13 mo.; spontaneous 53 mo.	Remission, ± proteinuria 65 mo.

* Time estimated from first appearance of edema. Although many observations were available for each patient, only those coincident in time with observations of Table III are recorded herein.

† Urinalyses were either semiquantitative (Addis) or qualitative. Decimalized data in columns 5–7 refer to semiquantitative excretions per 12 hour period. Proteinuria expressed in grams; RBC and cast excretions as millions per 12 hours. Qualitative analyses are usually given as ranges; RBC and casts as numbers per high-powered field of centrifuged sediment.

‡ Edema estimated on an arbitrary scale of none = 0 to extreme = 4+.

§ No autopsy.

‖ Anorexia, pallor and weakness for several months before edema.

¶ Postmortem: large (approximately twice normal weight) pale kidneys. Capsule somewhat adherent with a granular surface studded with petechiae. Cortico-medullary boundaries fairly well preserved. Most glomeruli were fibrosed; viable remainder evidenced marked proliferative response. Interstitial fibroses, small round cell infiltration, tubular dilatation, and atrophy were evident.

** Postmortem: right adrenal medullary hemorrhage. Kidneys twice normal weight; cut surface, loss of cortico-medullary zone. Moderate to marked epithelial hypertrophy of most glomeruli with beginning crescent formation and occasional partial hyalinization. Lipoid in tubular cytoplasm with some areas of vacuolization.

†† Postmortem: atheromatosis of aorta, and normal sized kidneys studded with petechiae. Poor cortico-medullary differentiation. Glomeruli showed all degrees of involvement; normal, increased cellularity and crescent formation to hyalinization. Hypertrophy and atrophy of tubules with some lipoid deposition and vacuolization of tubular epithelium. Arterioles showed varying degrees of intimal proliferation.

‡‡ Postmortem: *E. coli* bacteremia, peritonitis, pneumonia. Parathyroid hyperplasia and hypoplasia of bone marrow grossly evident. Kidneys were three-fourths of normal size, pale, easily stripping capsule and poor cortico-medullary differentiation on cut surface. Almost all glomeruli showed involvement varying from round cell infiltration and slight proliferation to complete scarring and hyalinization. No active crescent formation. Tubular degeneration and atrophy with isolated dilated, hypertrophied tubules evident. Fat largely in glomerular blood vessels.

§§ Postmortem: Parathyroid hyperplasia; slight atherosclerosis. *Staph. albus* cultured from pleural cavity; *Staph. aureus* from lungs and peritoneal cavity. Heart blood culture negative. Kidneys small and capsule slightly adherent. Small white granules on the surface. Cortical zone appeared well preserved grossly, but most glomeruli showed either hypercellularity or thickening, frequently with crescent formation. The tubules were generally atrophied with occasional necrosis of the epithelial linings.

RELATION OF SERUM NON-PROTEIN NITROGEN TO SIMULTANEOUSLY DETERMINED GLOMERULAR FILTRATION RATE IN CHILDREN WITH THE NEPHROTIC SYNDROME

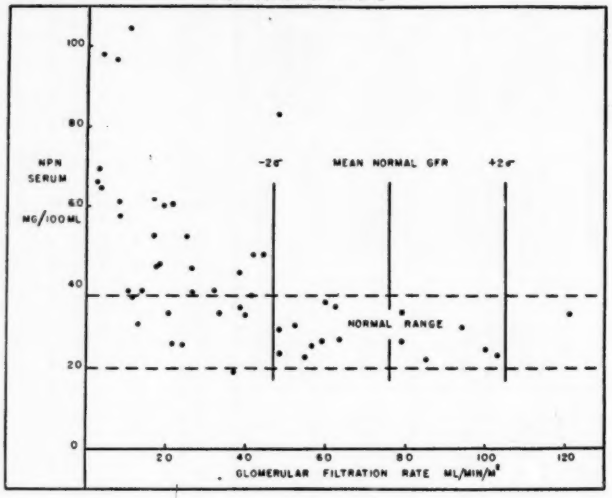

FIG. 1.

ACIDOSIS CONCOMITTANT TO REDUCED GLOMERULAR FILTRATION IN CHILDREN WITH THE NEPHROTIC SYNDROME

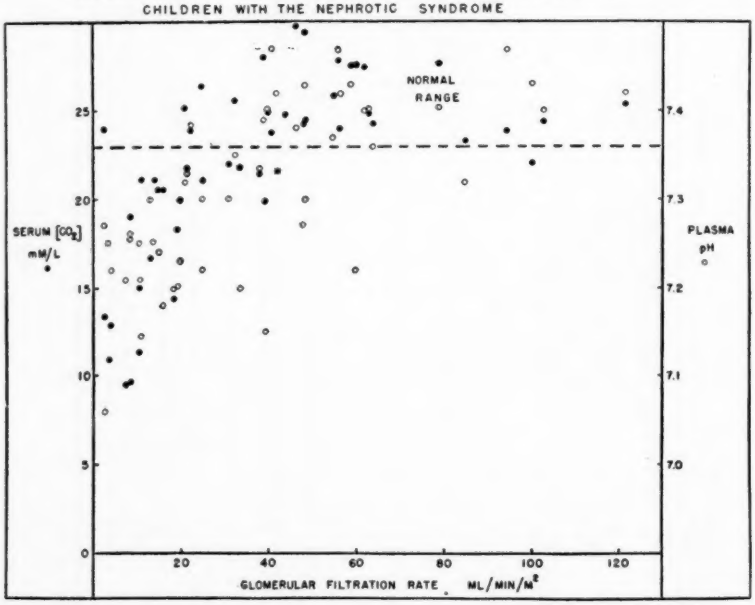

FIG. 2.

to the buffer base concept suggested by Singer and Hastings (37) (Figure 3). In approximately half of the patients, whole blood buffer base was estimated. In these instances, the derived values were slightly different; the trend and relationships were the same. It was evident that reduction of serum buffer base was usually associated with reduced GFR; however, reduction of GFR was observed occasionally without concomitant lowering of serum buffer base. Anemia, when accompanying azotemia and acidosis, completed a triad which indicated marked limitation of renal regulation and suggested a very poor prognosis. Anemia is rare early in the nephrotic syndrome. These three variables, rather arbitrarily related to each other, have been expressed as a ratio and correlated with GFR (Figure 4). The limitations of such an expression are obvious. In most instances, reduction in the arbitrary ratio accompanied roughly proportional reduction in GFR. However, one child (No. 24),[8] with moderate reduction in the arbitrary ratio after 17 months of active disease, appeared to be in clinical remission approximately one year later although functional data suggested residual renal damage. Several children with very low ratios have died (Nos. 5, 11, 18, 23, 26, 28); the others have persistent nephrotic symptoms and ample evidence of renal insufficiency.

Renal function data

The pertinent renal function data for each patient are presented in Table III and may be correlated with the clinical and biochemical observations in Table II. The validity of the "clearance" concept in the presence of nephron damage has been justifiably questioned (38–40). Some data on the response to adrenocorticotropic hormone (ACTH) therapy is included since ACTH may indirectly alter the metabolic activity of viable nephron cells (41, 42).[9] It can hardly be expected to restore destroyed, fibrosed or hyalinized tissue. Therapeutic response, therefore, affords one possible means of contrasting reversible impairment of function with irreversible destruction of parenchyma.

Glomerular filtration rate.

The distribution of values for GFR in relation to the duration of the disease is depicted in Figure 5. The course of certain patients is indicated. In

[8] Here and elsewhere, patients' numbers are as in Tables II and III.

[9] A detailed report concerning physiologic and clinical response to ACTH is in preparation.

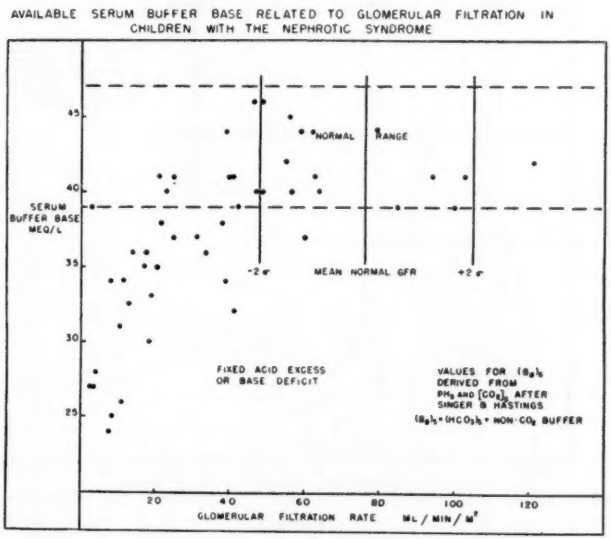

FIG. 3.

RELATION OF HEMOGLOBIN, SERUM NON-PROTEIN NITROGEN & BUFFER BASE TO GLOMERULAR FILTRATION RATE IN CHILDREN WITH THE NEPHROTIC SYNDROME

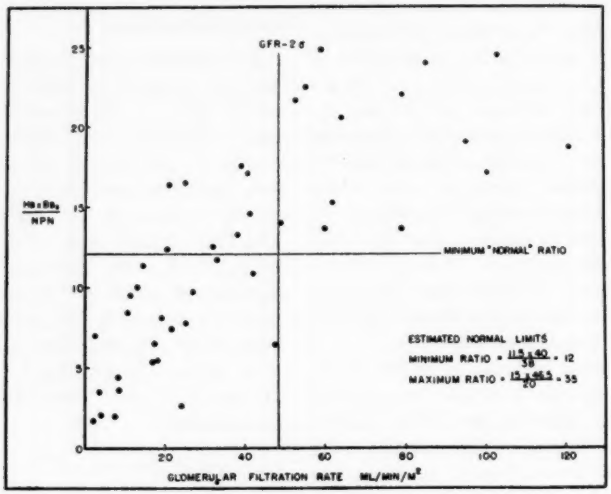
GFR-2σ
MINIMUM "NORMAL" RATIO
ESTIMATED NORMAL LIMITS
MINIMUM RATIO = 11.5 + 40 / 38 = 12
MAXIMUM RATIO = 15 + 65.5 / 20 = 35
Hb + BB / NPN
GLOMERULAR FILTRATION RATE ML/MIN/M²

FIG. 4.

RELATION OF GLOMERULAR FILTRATION RATE TO DURATION OF THE NEPHROTIC SYNDROME IN CHILDREN

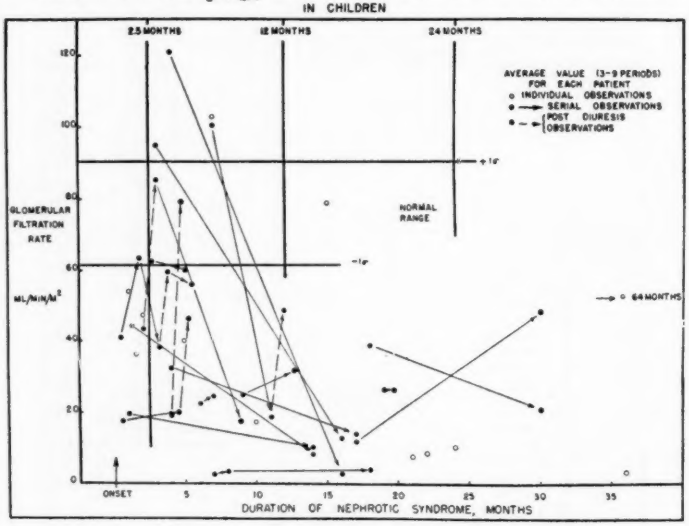

FIG. 5.

TABLE III

*Renal function data of children with the nephrotic syndrome**

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Patient data								Renal functions/M² (average)										V_D Thio.‡	
Case number	Patient	Determination number	Age (*years*)	Height (*cm.*)	Weight (*Kg.*)	Surface area (M^2)	Duration of disease (*months*)	GFR inulin *ml./min.*	GFR $Na_2S_2O_3$ *ml./min.*	RPF *ml./min.*	Est. extract. ratio†	RPF_e† *ml./min.*	TmPAH	$\frac{GFR}{RPF}$	$\frac{GFR}{RPF_e}$†	$\frac{RPF}{TmPAH}$	$\frac{GFR}{TmPAH}$	Liters	λe
Normal values:		25 Children (49)§ 1-5/12–10-1/12 61 Adults (22)		SA = 0.48–1.24 SA = 1.48–1.91		1.00 1.00		76 ± 15 75 ± 12	74(19)	364 ± 69 403 ± 78	0.89 0.90	410 ± 70 448	42 ± 10 47	0.21 ± .05 0.19 ± .02	0.19 0.17	9.0 ± 3.5 8.6	2.1 ± 0.9 1.7		
1	S. J. ♀	1	4-5/12	110.9	23.81	0.76	0.4	42	41	240	0.81	296	50	0.16	0.14	4.8	0.9	9.6	0.0012
		2	4-6/12	111.0	20.96	0.76	1.7	63	66	268	0.84	319	39	0.24	0.19	6.8	1.6	5.3	0.0121
		3	4-8/12	112.0	23.25	0.76	3.1	35	49	296	0.87	340	30	0.12	0.10	9.9	1.1	6.0	0.0134
		4‖	4-9/12	112.0	21.27	0.76	4.0	52	66	264	0.84	315	30	0.20	0.17	8.9	1.6	7.4	0.0085
		5	4-11/12	113.1	22.93	0.83	5.5	54	59	174	0.73	238	37	0.31	0.22	4.7	1.2	5.4	0.0128
2	C. P. ♀	1	1-4/12	81.0	10.24	0.44	0.6	10	22	125	0.63	199	14	0.09	0.09	9.2	1.1	3.8	0.0113
		2	1-8/12	81.0	10.32	0.44	4.5	19	21	85	0.52	163	8	0.23	0.12	10.2	2.6	3.5	0.0063
		3‖	1-8/12	83.0	8.86	0.43	5.2	43	49	215	0.78	276	22	0.21	0.16	9.7	1.9	2.4	0.0141
3	A. G. ♀	1	1-3/12	80.0	14.28	0.50	1.0		19	581	0.90	646	18	0.03	0.03	32.7	1.1	3.8	0.0171
		2	2-4/12	81.2	11.23	0.49	14.0	10	11	12¶	0.13	106	1**	0.80	0.10	12.9	6.5	2.4	0.0120
4	L. A. ♀	1	2-3/12	89.6	12.11	0.52	1.0	48	62	208	0.77	270	43	0.23	0.18	4.9	1.0	3.8	0.0136
5	R. S. ♂	1	6-6/12	123.0	33.90	0.98	1.0		44	545	0.90	605	45	0.08	0.07	12.0	1.0	11.1	0.0134
		2	7-8/12	125.1	29.19	0.95	14.0	9	8	20	0.20	96	1	0.44	0.09	27.9	14.3	10.1	0.0039
6	R. M. ♂	1	2-7/12	91.5	17.75	0.61	1.5		37	399	0.89	448	31	0.09	0.08	12.7	1.2	5.0	0.0084
7	B. D. ♀	1	1-8/12	87.0	14.75	0.50	2.0		48	303	0.87	349	31	0.16	0.14	9.7	1.5	6.5	0.0073
8	G. B. ♂	1	1-10/12	90.8	17.70	0.54	2.0	33	51									8.3	
		2‖	1-11/12	91.5	11.88	0.54	2.8	78	93	325	0.86	378	55	0.21	0.21	5.9	1.1	3.2	0.0191
		3	2-5/12	94.6	21.47	0.65	8.8	15	20	168	0.71	237	17	0.09	0.06	9.7	0.9	7.3	0.0101
9	H. M. ♂	1	3-4/12	105.4	17.86	0.71	2.5	57	69	270	0.85	318	52	0.21	0.20	5.2	1.0	5.5	0.0143
		2	3-8/12	106.1	24.63	0.73	5.0	55	65	198	0.76	260	38	0.28	0.21	5.2	1.2	8.2	0.0143
10	J. M. ♀	1	4-8/12	110.6	23.41	0.70	4.0		95	471	0.90	524	44	0.20	0.18	10.8	2.2	12.0	0.0108
		2	5-11/12	111.2	23.36	0.81	18.0	14	13	34	0.33	103	1	0.40	0.13	34.0	14.0	6.9	0.0087

TABLE III—*Continued*

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Patient data								Renal functions/M² (average)										V_D Thio.‡	
Case number	Patient	Determination number	Age (*years*)	Height (*cm.*)	Weight (*Kg.*)	Surface area (M^2)	Duration of disease (*months*)	GFR inulin *ml./min.*	GFR $Na_2S_2O_3$ *ml./min.*	RPF *ml./min.*	Est. extract. ratio†	RPF_e† *ml./min.*	TmPAH	$\frac{GFR}{RPF}$	$\frac{GFR}{RPF_e}$†	$\frac{RPF}{TmPAH}$	$\frac{GFR}{TmPAH}$	Liters	λe
Normal values:		25 Children (49)§ 1-5/12–10-1/12 61 Adults (22)		SA = 0.48–1.24 SA = 1.48–1.91		1.00 1.00		76 ± 15 75 ± 12	74(19)	364 ± 69 403 ± 78	0.89 0.90	410 ± 70 448	42 ± 10 47	0.21 ± .05 0.19 ± .02	0.19 0.17	9.0 ± 3.5 8.6	2.1 ± 0.9 1.7		
11	F. K. ♂	1 2	2-3/12 3-2/12	93.4 95.0	16.22 14.77	0.46 0.50	4.0 16.0	2	121 3	220 10	0.78 0.11	282 93	11 1	0.55 0.23	0.43 0.03	19.2 19.6	10.6 4.1	9.8 8.8	0.0133 0.0000
12	J. Ma. ♂	1 2‖	3-0/12 3-1/12	100.2 100.6	23.74 15.62	0.65 0.65	4.0 4.7	20 68	23 90	240 279	0.81 0.85	297 328	32 37	0.08 0.24	0.07 0.21	7.5 7.5	0.7 1.5	7.2 4.4	0.0097 0.0165
13	J. F. ♀	1 2	3-11/12 5-0/12	96.1 98.0	16.06 14.49	0.62 0.60	4.0 17.0	13	32 15	287 114	0.86 0.60	334 190	22 6	0.11 0.12	0.10 0.07	13.1 18.4	1.5 2.2	5.3 4.3	0.0104 0.0035
14	G. C. ♂	1	2-1/12	92.0	15.00	0.56	5.0	49	40	272	0.85	320	23	0.14	0.13	12.0	1.7	5.5	0.0059
15	R. G. ♂	1 2	3-1/12 3-3/12	90.1 91.0	15.81 14.61	0.54 0.56	6.0 7.5		23 25	178 258	0.73 0.83	243 310	12 30	0.13 0.10	0.09 0.08	14.6 8.6	1.9 0.8	5.6 4.8	0.0103 0.0113
16	P. N. ♀	1	2-8/12	91.5	15.07	0.52	6.0		103	442	0.90	490	38	0.23	0.21	11.7	2.8	6.0	0.0217
17	R. B. ♂	1‖ 2 3‖	2-1/12 2-5/12 2-6/12	85.5 88.0 89.3	13.33 18.71 13.37	0.52 0.55 0.55	7.0 11.0 12.0	92 19 51	96 20 47	239 136 127	0.78 0.65 0.63	307 209 202	20 9 9	0.43 0.14 0.30	0.33 0.09 0.25	12.3 14.5 14.5	6.2 2.1 4.6	6.2 3.7	0.0076 0.0152
18	E. D. ♀	1 2†† 3	2-6/12 2-7/12 3-10/12	91.0 91.0 92.9	20.20 13.91 17.55	0.56 0.58 0.60	7.0 8.0 22.0	4	2 4 4	88 111 12	0.53 0.59 0.14	167 187 88	21 23 1	0.03 0.04 0.35	0.02 0.02 0.05	4.2 4.7 14.4	0.1 0.2 4.6	3.7	0.0078
19	H. S. ♂	1 2	3-0/12 4-3/12	100.4 101.9	21.58 20.13	0.71 0.71	8.0 12.7	24 31	27 36	115 129	0.60 0.64	191 202	7 6	0.21 0.23	0.13 0.15	15.8 22.5	2.9 5.0	6.2 5.7	0.0105 0.0132
20	D. G. ♀	1	7-8/12	127.5	40.90	1.00	9.0		18	119	0.61	196	9	0.15	0.09	13.4	2.0	4.4	0.0206
21	L. C. ♀	1	3-11/12	99.0	15.86	0.64	15.0	59	99	308	0.87	353	49	0.19	0.17	6.3	1.3	3.3	0.0226

TABLE III—*Continued*

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Patient data								Renal functions/M² (average)										V_D Thio.‡	
Case number	Patient	Determination number	Age (*years*)	Height (*cm.*)	Weight (*Kg.*)	Surface area (M^2)	Duration of disease (*months*)	GFR inulin *ml./min.*	GFR $Na_2S_2O_3$ *ml./min.*	RPF *ml./min.*	Est. extract. ratio†	RPF_e† *ml./min.*	TmPAH	$\frac{GFR}{RPF}$	GFR † / RPF_e	$\frac{RPF}{TmPAH}$	$\frac{GFR}{TmPAH}$	Liters	λe
Normal values:		25 Children (49)§§ 1-5/12–10-1/12 61 Adults (22)		SA = 0.48–1.24 SA = 1.48–1.91		1.00 1.00		76 ± 15 75 ± 12	74(19)	364 ± 69 403 ± 78	0.89 0.90	410 ± 70 448	42 ± 10 47	0.21 ± .05 0.19 ± .02	0.19 0.17	9.0 ± 3.5 8.6	2.1 ± 0.9 1.7		
22	H. O. ♀	1 2	14-6/12 15-6/12	159.3 160.8	60.40 56.91	1.63 1.55	15.0 26.0	19	39 23	349 178	0.88 0.73	397 244	48 17	0.11 0.11	0.10 0.09	7.3 10.8	0.8 1.2	12.8 17.2	0.0198 0.0098
23	W. L. ♂	1	4-1/12	93.0	13.96	0.54	15.5		8	12	0.14	88	2**	0.62	0.09	5.1	3.1	6.2	0.0047
24	E. G. ♀	1 2	4-2/12 5-3/12	101.0 108.9	23.04 17.73	0.76 0.73	17.0 30.0	47	12 50	183 175	0.65 0.73	281 239	8 31	0.06 0.27	0.04 0.20	23.3 5.6	1.5 1.4	5.3 4.6	0.0159 0.0143
25	S. S. ♀	1 2‡‡	4-11/12 4-11/12	104.0 104.0	18.74 19.96	0.70 0.70	18.5 19.0	26 26	28	182 134	0.74 0.65	246 207	28 14	0.14 0.20	0.11 0.13	6.5 9.8	0.8 2.1	5.5	0.0098
26	B. S. ♀	1	5-1/12	101.0	22.05	0.71	20.0		10	22	0.21	104	3	0.48	0.10	6.7	3.2	6.1	0.0048
27	L. N. ♀	1	4-2/12	93.0	13.85	0.59	22.0		10	18	0.20	90	4	0.53	0.11	4.0	2.1	3.4	0.0104
28	E. M. ♀	1	4-6/12	94.0	20.86	0.62	35.0		4	19	0.20	91	3	0.19	0.04	6.7	1.3	7.6	0.0087
29	A. O. ♀	1	7-10/12	115.2	22.63	0.84	64.0	49	58	255	0.84	304	53	0.19	0.17	4.8	0.9	5.6	0.0131

* The order of patients and observations coincides with that of Table II—*e.g.*, according to duration of disease at time of initial study.
† Data in these columns based upon estimated extraction ratios; *cf.* text for limitations of such inference.
‡ Uncorrected for surface area.
§ Renal function studies on four "normal" children by our techniques gave similar values.
‖ Measurements obtained following ACTH induced diuresis.
¶ Despite infusion of minimal dose of PAH, serum levels of 10–11 mg. % were obtained. Estimate of PAH clearance may be invalid.
** One negative Tm period not included.
†† Values obtained when edema-free following paracentesis.
‡‡ Measurements obtained following ACTH, but before diuresis.

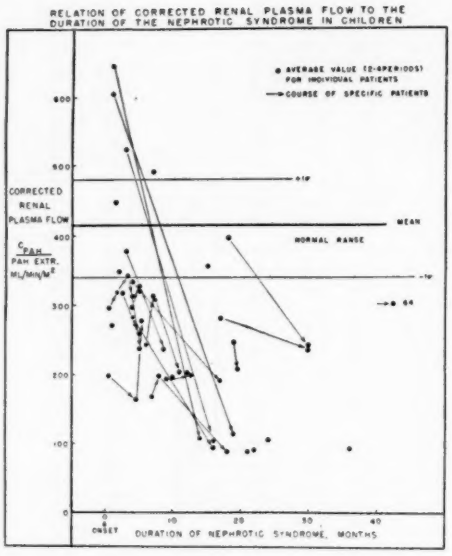
RELATION OF CORRECTED RENAL PLASMA FLOW TO THE DURATION OF THE NEPHROTIC SYNDROME IN CHILDREN
AVERAGE VALUE (2-3 PERIODS) FOR INDIVIDUAL PATIENTS
COURSE OF SPECIFIC PATIENTS
CORRECTED RENAL PLASMA FLOW
MEAN
NORMAL RANGE
ONSET
DURATION OF NEPHROTIC SYNDROME, MONTHS

FIG. 6.

general, glomerular function in the nephrotic syndrome varied with duration of the disease. Uniformly reduced filtration rates were noted in nine patients observed during the initial 2.5 months of the disease. Occasionally, supernormal values were noted in the following few months. More commonly, progressive reduction occurred.

Diuresis, which occurs spontaneously in many nephrotic children during the first weeks or months of the disease, usually was associated with improvement of GFR. Increased GFR and filtration fraction were not essential to initiate diuresis (42). In one instance (No. 1_2),[10] the initial diuresis was spontaneous; in others (Nos. 8_2, 12_2, $17_{1,2}$), it followed ACTH therapy. Following the initial diuresis and during the phase of recurring edema (Nos. 1_3, 8_3) reduced filtration was again noted. Four instances of supernormal filtration rates were observed after the first two months of the nephrotic syndrome. In one patient (No. 17_1), the observation was preceded by diuresis initiated with ACTH therapy. In others, (Nos. 10, 11, 16), no therapy had been employed. Subsequent determinations (Nos. 17_2, 10_2, 11_2) revealed striking reduction of filtration. An increase in filtration rate was observed twice in the same patient (Nos. 1_4, 17_3) following successive courses of treatment with ACTH with diuresis. Spontaneous or ACTH induced improvement of GFR after many months of active disease was also noted twice (Nos. 19_2, 24_2).

With continuation of the disease, progressive lowering of GFR was ultimately associated with renal failure. Extreme reduction in GFR was noted (No. $18_{1,2}$) for many months before clinical evidence of renal failure became apparent. Demonstration of diminished filtration after 17 months of active disease did not preclude apparent clinical remission, (No. 24). Subsidence of nephrotic symptoms and signs after a protracted course was usually characterized by persistent evidence of minimal inflammatory renal response and slightly reduced GFR (Nos. 17_3, 24_2, 29). Relatively early clinical remission was accompanied by normal GFR (No. 21).

Renal plasma flow

Unlike GFR, greater than normal values for apparent renal plasma flow were occasionally observed during the first months of the disease (Figure 6). Marked elevation of renal plasma flow was observed concomitantly with supernormal GFR (Nos. 10, 16) as well as with moderately (No. 5_1), or markedly (No. 3) reduced GFR. In several instances, elevation of the filtration rate was associated with diminution of renal plasma flow (Nos. 11_1, 17_1). It was evident that change in renal plasma flow could occur without significant variation in GFR (Nos. 1_5, 3, 9, 15, 18, 22). The reverse also was true (Nos. 1, 12, 17).

Following diuresis, either spontaneous (No. 1_2) or induced (Nos. 1_4, 17_3, 12_2), a definite increase in renal plasma flow usually was not observed. In one instance (No. 2_3), RPF was apparently doubled. While normal values were occasionally noted with apparent clinical remission (Nos. 12, 21), reduced renal plasma flow was also observed (Nos. 24_2, 29). Persistence of the nephrotic syndrome was usually associated with progressive reduction in both measured and "corrected" renal plasma flow (Nos. 5, 8_3, 13, 17_2, 18, 22).

Maximal tubular excretory capacity

Maximal tubular excretory capacity was usually within normal limits or was slightly reduced during

[10] Subscript refers to determination number of a given patient; in this instance, to the second renal function study of patient S. J.

RELATION OF MAXIMAL TUBULAR EXCRETORY CAPACITY TO DURATION OF THE NEPHROTIC SYNDROME IN CHILDREN

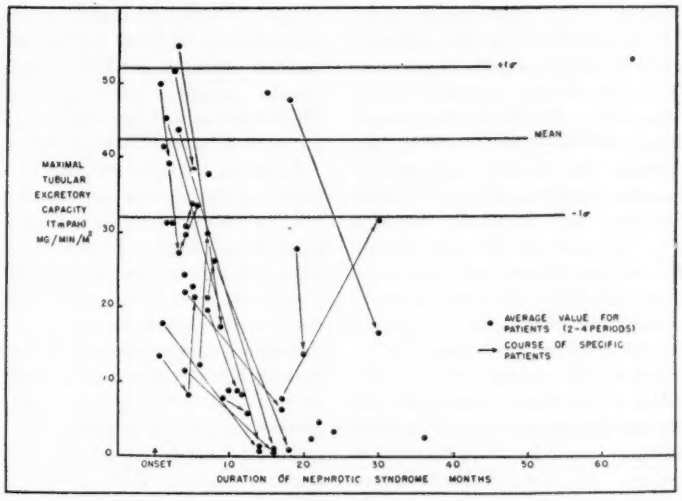

FIG. 7.

the first months of the disease (Figure 7). Neither spontaneous nor ACTH induced diuresis commonly was associated with significant alteration in this function (Nos. $1_{2,4}$, 12_2, 17_3) except in (No. 2_3). Early reduction in maximal tubular excretory capacity was apparently reversible, (Nos. 2_3, 15_2). With persistence of the disease, progressive reduction in Tm_{PAH} was observed. With clinical remission, despite a protracted course, normal values were observed (Nos. 21, 24, 29). Tubular reabsorption of PAH possibly accounts for the occasional observation of negative Tm periods (Nos. 3, 23) or apparent extreme reduction of effective tubular mass (Nos. 5, 10, 11, 18, 26–28).[11]

DISCUSSION

Marked distortion of the nephron and its supporting structures with extreme variation in architecture is not common in the early phases of the nephrotic syndrome in children (43, 44). In instances where death has resulted from intercurrent infection in the early months of the disease, the absence of striking morphologic changes, particularly of the glomerulus, has been evident (9, 11). Our data indicate a slight to moderate reduction of glomerular filtration early in the course of the nephrotic syndrome. Filtration is largely the resultant of hydrostatic pressure in one direction, oncotic and renal interstitial pressure in the other, and of the area over which the resultant pressure is exerted. Changes in effective renal arteriolar resistance may modify filtration (45, 46). The ratio of glomerular filtration to renal plasma flow (filtration fraction) defines the filtration equilibrium. Early in the nephrotic syndrome, the filtration fraction (FF) occasionally was observed to be essentially normal; rarely, it was markedly elevated; most commonly it was reduced (Figure 8). Renal plasma flow was usually reduced early in the disease, but filtration appeared to suffer greater proportionate reduction, although inconstant dissociation of the two functions was evident (Figure 9). The relationship between the two variables is illustrated by means of a correlation diagram of the sort employed by Goldring and his associates (47) and Smith (48). In this diagram, the elipse represents the perimeter of a normal surface within which 70% of normally correlated values would

[11] Despite rising serum PAH and inulin levels, consecutive periods commonly revealed little change in Tm although a slight tendency toward depression of simultaneous inulin clearance was noted.

be expected to fall. The statistical background for the elipse was calculated from the data of Rubin, Bruck, and Rapoport (49). Spontaneous or induced (ACTH) improvement of the nephrotic syndrome was usually associated with an increased filtration fraction. An absolute increase of GFR usually was responsible. Plasma protein concentration (or content) did not appear to undergo appreciable diminution with diuresis, and possibly increased. Therefore, further reduction of oncotic pressure accounting for increased filtration seemed unlikely. An increased filtration fraction associated with reduced afferent and augmented efferent arteriolar resistance has been observed with diuresis in nephrotic edema (42). Improved filtration during the nephrotic syndrome might result from decreased interstitial renal (subcapsular) pressure, alteration in membrane permeability, increase of the effective glomerular bed by restoration of normal circulatory channels, or by increase in glomerular pressure. It is not possible to choose between these alternatives at present. Since reduced GFRs were demonstrably reversible for variable periods of time, a disease process irreversibly obliterating glomeruli during the relatively early phases of the nephrotic syndrome seems unlikely. Persistence of the nephrotic syndrome with evidence of active renal disease for some period of time usually was associated with gradual reduction in all functional moities. When, in cases of the protracted nephrotic syndrome progressive diminution of filtration fraction was observed, associated changes in RPF/Tm_{PAH} and GFR/Tm_{PAH} suggested extensive nephron damage. These observations are consistent with those reported in glomerulonephritis (14, 15).

Increased maximal tubular excretory capacity following diuresis was occasionally observed in the first few months of the nephrotic syndrome. These observations suggest increased activity of the tubular cell. It has been demonstrated that the excretory capacities of the tubular cell may be altered under certain conditions (50). In either case, the concept of primary degenerative, irreversible change of the tubular tissue seems untenable. With clinical remission following many months of an active, persistent nephrotic syndrome, the effective tubular mass appeared to be essentially normal or only slightly reduced. With relapse, or progressive, active disease, reduction of Tm appeared to correlate with relatively symmetrical reduction of GFR (Figure 10). This phenomenon has been interpreted as evidence for ablation of functioning nephrons (48). The simultaneous appearance of biochemical renal insufficiency (Table II) tended to corroborate this interpretation. Reduction of

RELATION OF FILTRATION FRACTION TO DURATION OF DISEASE IN CHILDREN WITH THE NEPHROTIC SYNDROME

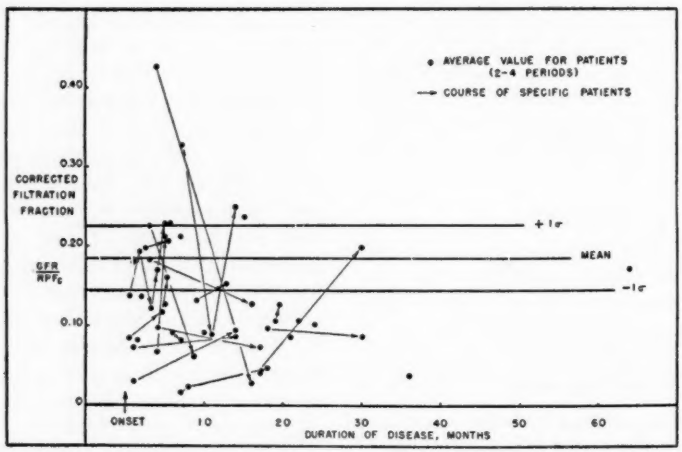

FIG. 8.

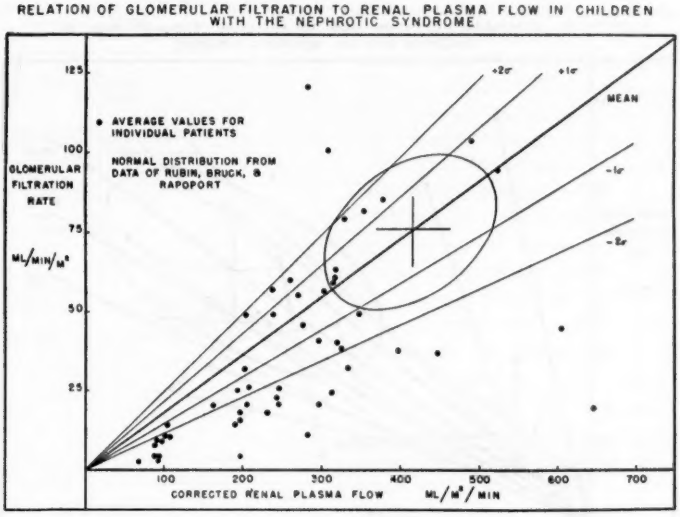

FIG. 9.

effective tubular mass, however, appeared disproportionately greater than diminution of renal plasma flow (Figure 11), suggesting hyperemia of the residual tubular tissue. In view of pathologic studies indicating distortion, vascular obliteration (51), and relative renal ischemia in advanced renal disease (15, 52), it seems likely that the apparent hyperemia is vicarious.

Neither the clinical observations nor the renal functional data clarify the etiology or pathogenesis of salient features of the nephrotic syndrome such as edema, hyperlipemia, hypoproteinemia or hypocalcemia. However, the data of this report form the basis for an hypothesis concerning the renal hemodynamic adjustments in the nephrotic syndrome in children (Figure 12). The validity of clearance measurements in the presence of nephron disease and at very reduced levels of renal function is uncertain and interpretation hazardous. With this reservation, further qualified by limitations of sampling and the necessity for protracted observation, it is suggested that the initial renal insult, which may be of immunogenic nature (53), involves the entire nephron in variable degree, with early alteration of its functional capacities. Preponderance of glomerular or tubular dysfunction may be observed. Initial reduction of GFR and FF may be contingent upon increased renal interstitial pressure, impaired glomerular membrane permeability, cortico-medullary shunts bypassing some glomeruli, or reduction of glomerular pressure. Diminished oncotic pressure would tend to improve glomerular filtration. Physiologic adjustment might attend reduction of subcapsular and increased intra-glomerular pressures. Constriction of the efferent arterioles, if associated with some dilatation of the afferent arterioles, reduced renal interstitial pressure and minimal alteration of the glomerular membrane, could theoretically induce supernormal glomerular filtration. Successful adjustment of the metabolic activities of tubular cells might result in diuresis and at least temporary remission. Recovery would follow successful, persistent functional reorganization of the nephron before irreversible anatomic damage was established. Failing functional reorganization, or possibly with recurrent stimulation of sensitized renal tissues by a primary etiologic agent, gradual distortion of the vascular architecture and dependent nephron structures characteristic of chronic renal disease might then initiate a vicious cycle of glomerular obliteration with diminished tubule blood flow and vascular shunting. Relatively increased blood flow adjacent to residual tubular tissue would thus represent

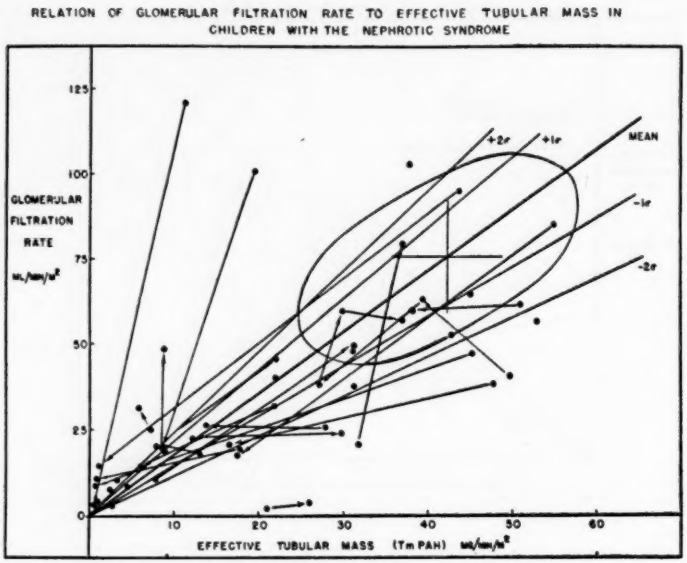
RELATION OF GLOMERULAR FILTRATION RATE TO EFFECTIVE TUBULAR MASS IN CHILDREN WITH THE NEPHROTIC SYNDROME
GLOMERULAR FILTRATION RATE
EFFECTIVE TUBULAR MASS (Tm PAH)
+2σ
+1σ
MEAN
-1σ
-2σ

FIG. 10.

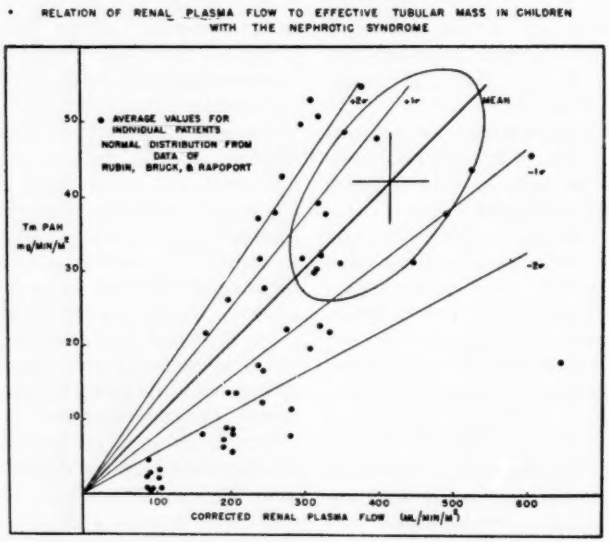
RELATION OF RENAL PLASMA FLOW TO EFFECTIVE TUBULAR MASS IN CHILDREN WITH THE NEPHROTIC SYNDROME
AVERAGE VALUES FOR INDIVIDUAL PATIENTS
NORMAL DISTRIBUTION FROM DATA OF RUBIN, BRUCK, & RAPOPORT
Tm PAH
CORRECTED RENAL PLASMA FLOW
+2σ
+1σ
MEAN
-1σ
-2σ

FIG. 11.

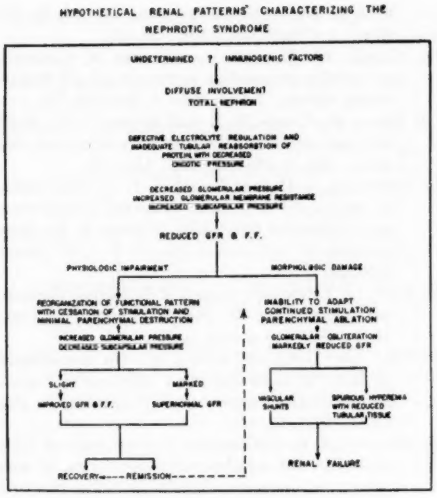

FIG. 12.

a spurious hyperemia and an indication of impending renal failure.

Since young children, unlike older patients, often recover following the nephrotic syndrome, the more favorable tissue reactivity of the young organism may explain the different course of this disease in these two age groups.

SUMMARY AND CONCLUSIONS

Twenty-nine children with the nephrotic syndrome, a fairly typical sample of 90 patients recently observed, have been studied by clinical, biochemical, and renal function techniques.

Conventional clinical criteria frequently proved unreliable early in the course of the nephrotic syndrome as indices of the functional status of the kidney or as guides to the subsequent course of the patient. The triad of anemia, acidosis, and azotemia, when correlated with a diminished GFR, indicated incompetence of the nephron for biochemical regulation and probable morphologic damage with poor prognosis.

Fluctuating changes in GFR, RPF, and Tm_{PAH} during the nephrotic syndrome suggested that variations in these measurements are a function of the severity and duration, rather than type, of disease. The biochemical and renal functional patterns indicate that reversible metabolic and hemodynamic rather than irreversible anatomic nephron damage frequently occurs. With progressive disease of long duration, markedly impaired renal function suggested that irreversible parenchymal destruction gradually supersedes the earlier physiologic changes, with culmination in renal failure.

The primary etiologic mecharism remains undetermined; however, the idiopathic nephrotic syndrome in children, whether called "pure or lipoid nephrosis," "mixed nephritis," or "nephrotic stage of subacute or chronic glomerulonephritis," appears to be a form of nephritis in which functional alteration and eventual anatomical changes indicate that the glomerulus is a primary site of the disease process. With inhibition of the unknown etiologic mechanism presumably before extensive anatomic damage has supervened, spontaneous or induced remissions may proceed to apparent recovery.

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STUDIES IN DISORDERS OF MUSCLE. VII. CLINICAL MANIFESTATIONS AND INHERITANCE OF A TYPE OF PERIODIC PARALYSIS WITHOUT HYPOPOTASSEMIA [1]

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INTRODUCTION AND REVIEW OF LITERATURE

The report by several investigators in 1937 and 1938 that the paralytic attacks in the disease, periodic paralysis, were associated with depressed serum potassium values led to the conclusion that the disorder is the consequence of some fault in the regulation of serum potassium levels (1–4). Ample support for such a concept appeared to be given by other observations. Thus, depletion of serum potassium in various other clinical circumstances, as in the recovery from diabetic acidosis and in the Addisonian patient overtreated with desoxycorticosterone acetate, is associated with flaccid paralysis. Again, it is known that hypopotassemia results in cardiac arrest in diastole and causes impaired contractility of muscle *in vitro*. Finally, the balance studies of Danowski and associates (5) showed that, in the patients they studied, the attacks of paralysis were preceded by the movement of potassium into the intracellular phase and that urinary potassium excretion as well as the serum potassium concentration decreased.

Talbot (6) in 1941 summarized the data concerning more than 400 cases of periodic paralysis. The disorder has been usually referred to as familial or family periodic paralysis. It seems best to us as it did to Talbot to call the disease periodic paralysis, specifying the hereditary nature of the disorder when it is genetically determined, and referring to the other cases as sporadic, associated with thyrotoxicosis or in other ways specifying the mechanism involved where it is known. Talbot concluded that the syndrome probably represented a clinical entity due to a single metabolic dysfunction.

In our study of a kindred in which 33 individuals with clinically typical periodic paralysis were known to have occurred, we observed no striking hypopotassemia during attacks in the patients whom we studied in detail. This led us to undertake a critical review of the data dealing with this disorder in the literature. A poor correlation between the onset and the severity of the attacks and the magnitude of the depression of serum potassium values has been noted and commented upon by several authors, including Talbot (6). Occasional instances of paralysis without depression of serum potassium levels have been reported (7). Furthermore, the levels of serum potassium found during attacks in patients with periodic paralysis usually have not been as low as those observed in patients who do not carry this genetic trait but who have hypopotassemic paralysis resulting from better understood metabolic abnormalities (3, 6, 8, 9). A corollary observation is the apparent lack of specificity of potassium in relieving or preventing the paralysis in certain patients with periodic paralysis in contrast to its excellent effect in the other metabolic hypokaliemias (1, 6, 10, 11).

It seems probable, therefore, that periodic paralysis as described in the literature is produced by more than one mechanism or that the serum potassium depletion is not the fundamental disorder. Such anomalies as abnormal storage of glycogen as well as other changes in intracellular potassium metabolism might be responsible for these vagaries of the serum potassium levels. The lack of complete similarity of the hereditary cases suggests that different genetic mechanisms are involved, most probably multiple mutations. These mutations can be understood best by studying them in kindreds where the expression of the same gene in many individuals may be observed. This disorder, like many other human hereditary disorders

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such as hereditary muscular dystrophy, hereditary blindness, hereditary deafness, etc., must be broken down into specific genetic entities before it can be analyzed and interpreted adequately.

An examination of the genetic literature illustrates the diversity and variety of expression of periodic paralysis. The trait has been reported to be a dominant with skipped generations (12, 13), a complete dominant (14, 15), a recessive (6), and a sex-linked recessive (16). The gene has been reported as manifesting incomplete penetrance in females (6) and as having complete penetrance in both sexes (15). The expressivity may range from a slight recurrent weakness in one limb, as reported in one family (10), to paralysis so severe that nearly the entire body is paralyzed, in other families (13). Again, the paralysis may be so extreme in some cases as to cause death (13, 14).

The duration of an attack in the members of some families may range from a few minutes to several days while in other families an individual may never be affected for more than a few hours (6). In certain families the attacks are rare, occurring once or twice in a lifetime or two or three times in a year or in a decade (6). In contrast, a case has been reported in which the attacks occurred daily (17). The onset has ranged from four to 35 years in one family (15), was consistently at 18 or 19 years of age in another (14) and, in yet another, the condition was evident in infants less than a year old (18). The attacks have been reported to cease in one family after the fourth decade (15).

Periodic paralysis has been observed in association with other diseases. One family was reported to have migraine as well as periodic paralysis (13) and another family with the trait had migraine and muscular atrophy that developed after years of attacks of paralysis (19). Others report the association of periodic paralysis with exophthalmic goiter (20–22). The transition of periodic paralysis to spinal muscular atrophy also has been described (23).

Many diverse clinical observations and even more diverse interpretations of these findings have been reported. Attacks have been thought to be precipitated by local application of cold (17), by glucose (5, 6, 24, 25), by water diuresis (26), and by insulin (2, 24, 25). In other cases, none of these was effective in bringing about an attack (10, 25, 26). Potassium chloride, thyroid extract (25), cholinergic drugs (26), and adrenal extract (2) have all been used effectively and ineffectively as therapeutic agents. Reports concerning potassium, sodium, phosphate, and magnesium ion levels have been conflicting (1, 2, 5, 7, 10, 22, 27, 28).

It has been postulated that the defect is related to the central nervous system, to the permeability of muscle cells, to hepatic or muscular glycogenesis or to a chronic deficit of potassium in the muscle (10, 28).

In addition to the analysis of the genetic pattern in this kindred we have made certain clinical and laboratory studies on some of these patients. We have studied methods of precipitating attacks, the effect of administering glucose or glucose and insulin, the effect of exercise and of enforced rest, and the correlation of these factors to serum potassium, sodium, magnesium, phosphate and blood glucose levels. Gross and microscopic examination of biopsy material from skeletal muscle as well as determination of its glycogen content also have been carried out.

METHODS

Pedigree, genetic and clinical data were secured by interviews with the members of the kindred. The trait in this kindred is of such a nature that it is readily identified by the members of the families, and their reports of its occurrence have been consistently accurate in those patients whom we have examined. Where it was necessary to obtain information regarding individuals who are now dead, a check was made from as many different sources as possible in order to be sure that the data were accurate. Blood types and ability to taste for phenylthiocarbamide (PTC) were determined for use in linkage studies.

Five patients were studied in detail on the metabolic ward of the laboratory. A full history, physical examination and clinical laboratory investigation, as well as certain special determinations as summarized in the introduction, were carried out. Blood glucose values were determined by the method of Nelson (29) on alkaline zinc-barium filtrates of whole blood collected in a fluoride-oxalate anticoagulant. Serum potassium and sodium were measured on a flame photometer (30). Serum magnesium values were estimated by the colorimetric method of Kunkel and coworkers (31), muscle glycogen by the method described by Good and colleagues (32). The remaining chemical and hematologic methods used have been described in detail in other papers of this series and will not be reviewed here (33, 34).

CLINICAL DESCRIPTION OF THE TRAIT

The clinical manifestations of the trait as observed in this kindred consisted of episodes of weakness or flaccid paralysis. Such attacks began and ended insidiously. They lasted from a few minutes to many hours or even several days in rare instances. In severe attacks all of the skeletal muscles were involved except those of facial expression, mastication, deglutition, speech, and respiration (diaphragm). Respiratory embarrassment of a severe character was not observed in any of these patients. In milder episodes localized areas were sometimes involved, usually the large muscles of the legs, arms or back, although an occasional individual had attacks in which only local involvement of the hands occurred. The pattern was usually symmetrical on both sides of the body. The paralysis was flaccid, deep tendon reflexes were absent and electrical excitability of the muscle was greatly reduced or lost.

The attacks began most frequently in the early morning and not infrequently were present on awakening. Activity tended to decrease the degree of weakness present if paralysis did not preclude movement. On the other hand, strenuous exercise which was followed by complete inactivity very frequently initiated an episode. This was the most reliable manner of initiating attacks in the patients whom we studied. Paralysis did not occur while the patient continued to be active but the more strenuous the activity and the more complete the ensuing rest, the more probable it was that a severe attack would result. The patients consistently reported that they could "fight off" an incipient attack by persistent activity. This, it may be noted, is the exact converse of the effect of activity and rest in myasthenia gravis.

A number of other factors also appeared to play a role in the precipitation of attacks. A heavy meal or, less frequently, the ingestion of a large amount of carbohydrate, particularly if taken before bed time, sometimes preceded an attack on the ensuing morning or day. No patient reported attacks precipitated by high carbohydrate intake during the preceding two hours.

Some patients reported no difficulty from food ingestion regardless of amount. Still other periods of recurrent severe episodes occurred without any apparent relation to the activities of the patient. These were just as severe as those having apparent relationship to preceding exercise or other factors which the patient regarded as responsible for the attack.

During the attacks there were no symptoms other than those which related to the weakness itself, except that during severe paralysis marked perspiration usually occurred. That this may precede the paralysis and possibly be a factor in its development was suggested by the drenching night sweats which frequently preceded morning attacks. One patient reported that profuse sweating preceded most of his severe attacks. On the other hand, the profuse perspiration induced by heavy work in hot weather does not induce paralysis unless it is followed by more or less complete inactivity.

The initial attacks occurred in the first year of life and the parents who were well acquainted with the disorder were able to recognize the presence of the trait regularly during the first months of life. The episodes tended to be most frequent during childhood and adolescence, gradually decreasing in severity and frequency with advancing age although they still occurred in aged individuals. There was a moderate degree of variability in the severity of the syndrome from patient to patient, some being only occasionally inconvenienced, others having attacks of such severity that they must miss work on the average of one day per week. In the individual patient as well, a long period during which little trouble was experienced might be followed by frequently recurrent attacks for periods lasting a few days or many months. Such periods were often associated with continued emotional stress.

On physical examination two observations could be made almost routinely even though the patient felt that he was normal at the time. A slight but definite degree of weakness was present in the extensors of the arms and legs which was sufficient to show up in sharp contrast to the good strength present in the flexors. These patients presented the appearance of muscular, hearty individuals, a fact which had caused certain of the male members no end of difficulty in dealing with the armed services where weakness in an apparently muscular individual is presumed to be evidence of laziness. The muscular appearance was particularly marked in the calf muscles and extensors of the arms, and

was indistinguishable clinically from the pseudohypertrophy of childhood progressive muscular dystrophy, as previously discussed by one of us (35). Pathologically the findings are very different from those observed in the pseudohypertrophy of dystrophy, as will be described below.

The syndrome appears in both sexes. The symptoms are usually more severe in the males, possibly because of the somewhat more vigorous activity in which they engage and the relationship of exercise to the episodes of paralysis outlined above.

LABORATORY STUDIES

No significant laboratory abnormality has been observed in the five male patients whom we have studied in detail except for the occurrence of high serum uric acid levels in two. No history suggestive of gouty arthritis was obtained on these nor on other normal or abnormal members of the group. Table I is a summary of the routine and certain special determinations which were performed. The fasting serum potassium values, taken at times when no significant degree of paralysis existed, were normal. Those presented are representative figures taken from a large series of determinations, none of which was abnormal. The normal creatinine excretion values are consistent with the apparently normal muscle mass. Creatine excretion was insignificant. Volumes of packed red cells ranging about 50 per cent correspond to normal male values for this area which is over 4,000 feet above sea level.

The results of some of our attempts to induce attacks by the administration of glucose or glucose and insulin are summarized in Table II. None of these was successful in producing a significant change in the degree of disability present at the start of the tests although serum potassium levels of about 3.1 meq./L were observed on several occasions.

Simultaneous urine collections showed a marked drop in the excretion of potassium, as is regularly observed during the hypopotassemia following carbohydrate or insulin administration. Thus the metabolic picture observed by Danowski has been reproduced without precipitating an attack of paralysis. The time relationships are shorter which possibly is a significant factor in the problem. No exactly similar experiments have been carried out in other cases of periodic paralysis as far as the authors are aware and we have not had an opportunity up to the present to duplicate the observation in a patient showing definite hypopotassemia during spontaneous attacks. It is noteworthy, however, that in nearly all of the previously reported patients in whom carbohydrate or insulin appeared to have been related to attacks, the paralysis developed after a delay of six or more hours and not at the time of maximum hypopotassemia. We observed no significant increase in attacks of paralysis in our patients during the 24 hours following these experiments.

Glucose tolerance was certainly not impaired in any of these tests. The rather low 30 and 60 min-

TABLE I

Serum, whole blood and urine studies in hereditary periodic paralysis

	G. D. K. 10 Male	Bu. R. 19 Male	Bl. R. 22 Male	T. K. B. 23 Male	M. M. B. 27 Male
Potassium, meq./L	5.7	4.2	4.0	4.2	4.0
Sodium, meq./L		144	141	138	144
Magnesium, meq./L			2.0		2.1
Calcium, meq./L				5.3	5.5
Phosphate, mg./100 ml. as P			4.5	3.3	3.1
CO_2 combining power, mM./L		25.9		26.3	26.1
Chloride, meq./L	104	107			103
Uric acid, mg./100 ml.		7.8	5.5	7.9	4.8
Total protein, gms./100 ml.		8.9	7.5		7.9
Albumin, gms./100 ml.		6.6	5.2		5.9
Fasting blood sugar, mg./100 ml.	78	70	72	72	64
Blood urea nitrogen, mg./100 ml.	10	12	15	14	12
Routine clinical urine examination	Normal	Normal	Normal	Normal	Normal
Creatinine, mg./24 hrs. urine	754	1,525	1,366		1,459
Creatine, mg./24 hrs. urine as creatinine	70	200	0		73
Hct., % RBC	42	52	54	49	47
WBC in thousands/mm.^{3}	9.0	6.8	8.6	8.3	7.4

TABLE II

Blood sugar, serum potassium and serum phosphate values after oral or I.V. administration of glucose with or without insulin

Patient and procedure	Time in minutes.....	0	30	60	90	120	150	180	240
T. K. B. 96 gms. glucose I.V.	Blood glucose, mg./100 ml.	74	234	48	39		62		
	Serum potassium, meq./L	4.2	4.1	4.1	4.1		3.9		
	Serum sodium, meq./L	140	134	137	138		137		
	Serum phosphate, mg./100 ml. as P	3.7	2.8	3.0	3.1		3.8		
T. K. B. 100 gms. glucose orally	Blood glucose, mg./100 ml.	72	79	88	72	85		68	82
	Serum potassium, meq./L	4.7	3.6	3.6	3.7	3.7		4.2	4.0
	Serum phosphate, mg./100 ml. as P	4.2	3.5	3.5	3.6	3.7		3.9	3.9
M. M. B. 100 gms. glucose I.V.	Blood glucose, mg./100 ml.	53	207	164	76	34		57	62
	Serum potassium, meq./L	4.8	4.0	3.5	3.5	—		3.5	3.7
	Serum phosphate, mg./100 ml. as P	3.4	3.1	3.1	3.4	4.0		4.3	4.4
M. M. B. 200 gms. glucose orally	Blood glucose, mg. %	64	107	76	67	82		72	77
	Serum potassium, meq./L	4.0	4.1	4.5	4.4	4.1		3.7	4.4
	Serum sodium, meq./L	144	143	142	142	142		141	139
	Serum phosphate, mg./100 ml.	3.6	3.2	3.1	3.2	3.3		3.5	3.6
Bl. R. 35 gms. glucose I.V.	Blood glucose, mg./100 ml.	72	170	52	54			74	
	Serum potassium, meq./L	3.8	3.4	3.7	3.9			4.3	
	Serum phosphate, mg./100 ml. as P	3.6	3.4	3.6	3.5			4.1	
Bl. R. 100 gms. glucose and 25 units insulin I.V.	Blood glucose, mg./100 ml.	106	141	86	80		64		
	Serum potassium, meq./L	3.9	3.6	3.1	3.4		3.7		
	Serum magnesium, meq./L	2.0	1.9	1.6	1.9		1.9		
	Serum phosphate, mg./100 ml. as P	4.6	3.9	3.6	3.8		4.3		
Bu. R. 200 gms. glucose and 30 units insulin I.V.	Blood glucose, mg./100 ml.	70	187	14	25	39	53	57	
	Serum potassium, meq./L	3.7	3.1	3.1	3.1	3.4	3.6	4.2	
	Serum phosphate, mg./100 ml. as P	5.0	3.2	3.4	3.3	3.8	4.3	4.7	

ute blood glucose levels after oral administration might suggest either impaired absorption or increased glucose tolerance. Intravenous glucose tolerance tests using one-half gram of glucose per kilogram of body weight showed normal curves in three patients and no other evidence of altered carbohydrate metabolism could be obtained. During episodes of mild and severe paralysis we have obtained repeated serum samples which have been analyzed for sodium, potassium, and magnesium, as well as a number of other substances. All of these values have been consistently normal. A few are presented in Table III; the values during attacks are those obtained at the time paralysis was most severe.

Additional comment about the serum potassium values seems in order because of the many previous observations of depressed levels. On two occasions we have obtained multiple samples at approximately 15 minute intervals during artificially induced attacks (exercise followed by enforced rest). The serum potassium values on these occasions varied over a narrow range (0.4 meq.) and showed no consistent pattern or correlation to the degree of paralysis at the time the serum was obtained. Electrocardiograms taken during the attacks showed no change from the control tracings.

We have observed no values below 3.5 meq./L except in association with the glucose-insulin infusions. All of the values were in the same range as observed in normal individuals in our laboratory.

Oral therapy with potassium chloride, either prophylactically or during an attack, failed to influence the course of the disorder. However, we have not had the opportunity to observe the effect of potassium given orally or intravenously during an episode of severe paralysis of more than about one hour's duration, and the possibility, albeit im-

TABLE III

Serum electrolyte values before and at the time of maximum paralysis in four patients

Patient	Serum potassium *meq./L.*			Serum magnesium *meq./L.*	
	Before attack	During attack	Change	Control	During attack
G. D. K.	5.7	5.7	0.0		
Bu. R.	4.2	5.0	+0.8		
Bl. R.	3.9	3.7	−0.2	2.0	2.0
	4.2	4.2	0.0		1.95
	5.4	5.4	0.0		2.2
	3.6	4.0	+0.4		
	3.8	4.2	+0.4		
T. K. B.	4.2	3.5	−0.7		

probable, exists that the effect might be different under such circumstances. Actually all of the group who have taken potassium prophylactically by mouth dislike the medication and report that they have experienced just as many and as severe attacks while receiving such therapy as at other times. In addition, a number have experienced nausea, dizziness, and a vague lack of well-being while taking potassium, and all have discontinued it within a few weeks of its first administration.

PATHOLOGY

Three patients submitted to biopsy of their enlarged gastrocnemius muscle mass. Grossly the muscles appeared essentially normal, contracted on mechanical stimulation, and showed no evidence of fatty replacement or of fibrosis.

Microscopically, characteristic degenerative changes were recognized in the muscle of all three patients but the degree of change varied widely. In the sample which showed the slightest degree of alteration, most of the fibers were well preserved and their strial markings were clearly defined but their diameters exhibited greater than normal variation. Some fibers were unusually slender and were stained more strongly with eosin than were the fibers of normal size. Irregular clusters of nuclei appeared along the more slender portions and, in a few foci, appeared to replace the substance of fibers. In other places, portions of fibers were missing and the spaces were partly filled by pycnotic nuclei, monocytes, polymorphonuclear neutrophils, fibrocytes and a few well preserved muscle nuclei. The most characteristic feature was the presence of vacuoles near the centers of a few fibers and appearing at sites of fusiform enlargement (Figure 1). The largest of these were round, fusiform or oval, occupied the entire diameter of the fiber and were limited laterally only by sarcolemma. The smaller spaces appeared to arise within sites of basophilic degeneration where longitudinal and transverse striae were absent and acidophilic granules appeared. The vacuoles occurred as multiple foci of rarefaction, sometimes lined up along the axis of the fiber like a string of beads. The larger vacuoles, apparently formed by coalescence of several small ones, often contained acidophilic granules and occasionally were filled with faintly basophilic coarse granules. A few contained one or two pycnotic nuclei.

In the sample of muscle exhibiting the most advanced degree of degeneration, very few muscle fibers appeared normal although many retained their striae. Irregularly swollen, hyalinized fibers alternated with degenerated fibers containing vacuoles of all sizes and shapes. Areas of segmental disintegration were often associated with an increased number and pycnosis of nuclei. Some portions of fibers were represented by amorphous acidophilic debris.

Frozen sections stained with Sudan IV and hematoxylin failed to show abnormal quantities or distribution of lipids.

Sections of muscle, fixed in absolute alcohol and stained by Best's carmine method, showed marked irregularity of distribution of glycogen granules. Many coarse granules were found in those portions of the fibers which appeared swollen and degenerated. Some of the vacuoles contained amorphous masses and coarse granules which were carmine-positive but many of the vacuoles were entirely empty or contained unstained debris.

Two of the biopsy specimens were analyzed for total glycogen content. The values, together with those of two control specimens from human material, are listed in Table IV. It will be noted that all of the values fall within the normal range for muscle glycogen.

TABLE IV

Glycogen content of muscle

Patient	Muscle	Glycogen as gms. glucose/100 gms. tissue
M. M. B.	Gastrocnemius	1.01
Bu. R.	Gastrocnemius	0.46
Control 1	Rectus Abdominus	1.33
Control 2	Rectus Abdominus	0.76

FIG. 1a.

FIG. 1b.

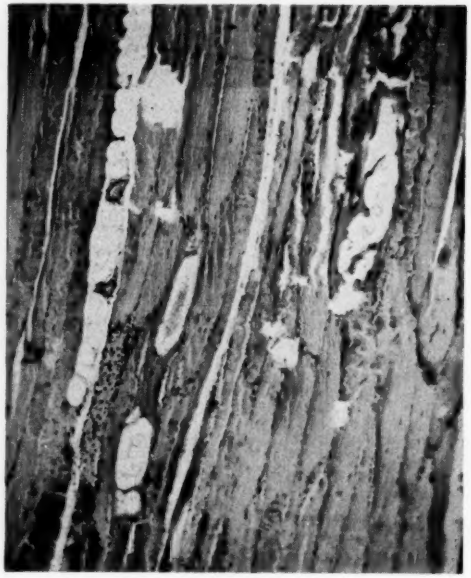

FIG. 1c.

FIG. 1. THREE PHOTOMICROGRAPHS ILLUSTRATING THE MICROSCOPIC CHANGES FOUND IN BIOPSY SPECIMENS FROM THE GASTROCNEMIUS MUSCLE

Note the central location of the vacuoles in (a) both in longitudinal and cross sections and the irregular size of the muscle fibers. The more extensive changes in (b) and (c) are apparent, some fibers showing almost complete replacement. The sections were stained with hematoxylin and eosin and are magnified about 78 diameters.

FAMILY HISTORY

Figure 2 is a pedigree chart of the kindred reported in this study. I-1 and I-2 migrated to Utah in 1854. They had three daughters (II-1, 2, 3). II-1 died at the age of 13. III-8 and 9, daughters of II-3, state that their mother was affected with periodic paralysis and that she told them that her mother (I-1) and sister (II-2) were also affected. II-2 had five children all of whom are now dead. Interviews with their children, or with other members of the family who were in a position to know of their condition, indicated that none of the five children nor their descendants was affected with periodic paralysis. The condition of II-1 is not known.

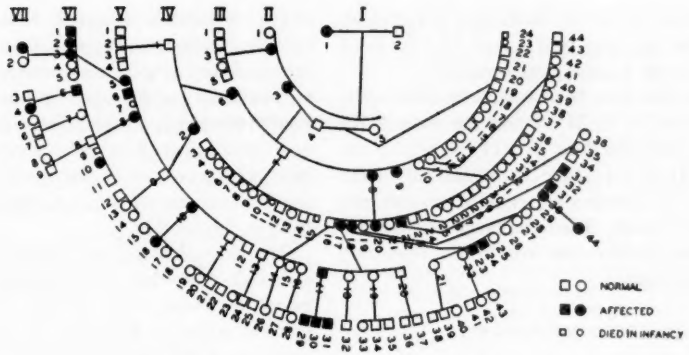

FIG. 2. PEDIGREE CHART OF KINDRED
Note that only affected individuals transmit the trait.

The husband of II-3 was a polygamist. He had ten children by his second wife (II-5) none of whom was affected with periodic paralysis. With the exception of I-1 and II-2, all of the known cases of periodic paralysis in this kindred descended from II-3. Nearly all members of the kindred are now living in Utah.

INHERITANCE

Examination of Figure 2 shows that the trait is transmitted through one family line and that it never occurs in a child unless it has also occurred in a parent. Table V shows the numbers of normal and affected individuals by sex in the families in which the trait occurred. It includes all of the known offspring of individuals who have periodic paralysis in this kindred. Table VI compares the totals of Table V to the expected results if the trait is inherited as a dominant with complete penetrance.

It is apparent that the trait occurs in approximately the same frequency in males and females and that approximately one-half of the children of an affected parent show the trait. This suggests that in this kindred, periodic paralysis is attributable to a simple dominant gene having complete penetrance. The deviation from the expected incidence is very slight. Since no two individuals showing the trait and no two relatives have married, affected individuals must be presumed to be heterozygous (Pp). The crosses, therefore, which have produced affected children, are back crosses ($\mathrm{Pp} \times \mathrm{pp}$) and should produce one-half affected and one-half normal children. Since we have no homozygous individuals, there is no way of deter-

TABLE V

Segregation of children of 16 individuals affected with periodic paralysis

Family		Affected		Normal		Condition unknown
Generation	Number	Male	Female	Male	Female	
I	1	—	2	—	—	1
II	2	—	—	4	1	
II	3	—	3	2	4	
III	6	—	1	1	—	
III	8	1	3	3	2	
IV	2	1	3	3	—	
IV	17	1	—	1	2	
IV	18	2	—	—	3	
IV	22	2	1	—	—	
V	5	1	2	—	2	
V	8	1	1	2	1	
V	10	—	1	—	3	
V	17	3	—	—	—	
V	30	—	1	—	—	
VI	3	—	1	—	1	
VI	6	—	1	1	—	
Totals		12	20	17	19	1

TABLE VI

Statistical comparison of data on this kindred with the theoretic behavior of a dominant trait

	Observed	Calculated	Deviation	S.E.	D./S.E.
Affected	32	34	2	4.123	.485
Normal	36	34			

mining whether or not the dominance is complete. It is possible that the trait would be expressed differently in the homozygous condition.

Blood samples have been taken and determinations made for A, B, M, N and the various Rh antigens. Taste reactions to PTC were also determined. It was possible to obtain data from nearly all of the families segregating for paralysis who are now living. The data, however, were insufficient to warrant any conclusion regarding linkage relationships.

EUGENIC IMPLICATIONS

In this kindred, periodic paralysis can be detected readily by members of the families concerned. Each individual showing the trait is heterozygous and stands an equal chance of transmitting or not transmitting it to each of his children. The trait can be eliminated only if each affected individual refrains from having children. Unaffected individuals do not transmit the trait.

DISCUSSION

A number of unanswered problems remain concerning the disorder which we have observed in this group of people. Clinically the process is nearly identical with previously described cases of periodic paralysis whether hereditary or apparently sporadic. On the other hand, the failure to demonstrate any relationship of the attacks of paralysis to serum electrolyte concentrations, particularly the absence of hypopotassemia, sets the group apart from the best studied cases reported in the literature. Were it not for the defects which were discussed earlier, in the concept that serum potassium depletion is the fundamental mechanism leading to the paralysis in other cases, it might be concluded that we have been dealing with an entirely different disorder. This is hardly justified, however, in the light of the variability of the previously reported cases and the clinical indistinguishability of this syndrome from them. It is possible that all types of periodic paralysis are due to a single pathogenetic mechanism the nature of which has not been identified. However, the evident hypopotassemia seen in other patients makes this rather improbable, and therefore it is more likely that there are two or more types of periodic paralysis.

The frequently reported relationship of attacks to carbohydrate ingestion led us to postulate the possibility of some abnormality in the immediate availability of glycogen in muscle for the production of energy. Our studies to date have yielded no evidence to confirm such a theory; instead they have only served to emphasize the poor relationship between carbohydrate administration and attacks in this kindred.

The unusual pathologic picture of vacuolization of muscle fibers has been observed before, as far as we are aware, only in acute traumatic or infectious injury of muscle or in experimental obstruction of the deep lymphatics. The lack of inflammatory cells or evidence either clinically or pathologically of lymph stasis in these patients is not consistent with such an explanation here, however. A somewhat similar but more severe lesion has been observed in glycogen storage (von Gierke's) disease in children. The possibility that the disorder which we have described represents a related phenomenon in which only the muscle was involved seemed reasonable. However, our inability to demonstrate excess total glycogen or microscopic distribution of glycogen in the muscle fiber characteristic of that disorder makes this highly improbable.

The clean-cut genetic pattern of dominant inheritance with complete penetrance which we have found is in contrast to the findings in some of the previously reported families. This would appear to be additional evidence of the multiple character of the genetic mechanisms which result in the clinical syndrome of periodic paralysis. However, as we have emphasized previously (36), it is difficult to obtain reliable genetic data unless many of the normal and abnormal individuals are examined. In many published reports this was not done.

CONCLUSIONS

1. A study of periodic paralysis occurring in 33 individuals in seven generations of a single kindred is reported.

2. Clinically the disorder begins in infancy with recurrent attacks of weakness or flaccid paralysis. Some extensor weakness and enlargement of muscles is evident at all times.

3. The attacks are most consistently produced by vigorous exercise *followed* by complete rest.

4. No correlation of the attacks with the serum potassium or other electrolyte concentrations could be established.

5. An unusual pathologic picture of vacuolization of scattered muscle fibers was found in biopsy material. Neither fat nor glycogen could be demonstrated in the vacuoles and it is presumed that they contained only edema fluid.

6. The pattern of inheritance is that characteristic of a simple mendelian dominant trait with complete penetrance.

ACKNOWLEDGMENTS

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RESPIRATORY REGULATION DURING EXERCISE IN UNCONDITIONED SUBJECTS [1]

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INTRODUCTION

The regulation of breathing during muscular exercise has been the subject of much work and speculation. Most of the experience has been gained with subjects in good physical training. In general, blood chemical changes in these subjects have not been great during exercise, and it has been difficult to account for the amount of ventilation on the basis of these changes and other known respiratory stimulants. The present study was done on untrained subjects in only fair physical condition. It seemed likely that the coordination of various respiratory drives would be less smooth in untrained subjects than in trained persons, so that the part played by different stimuli might become more obvious. The conduct of such studies has been simplified by the recent development of techniques for recurrent sampling of arterial blood during the course of exercise. Nearly all past studies have had to rely on indirect means of estimating arterial blood changes.

METHODS

The subjects were 11 male physicians and medical students in normal health but not in physical training. They ranged in age from 22 to 35 years and in surface area from 1.86 to 2.12 sq. m. They were not fasting and came to the experiment directly from their work. Arterial blood samples were taken from the brachial artery through an in-lying Cournand needle attached by flexible plastic tubing to a series of three way stopcocks. Blood was taken into syringes wet with heparin. For pH determinations, sodium fluoride was added as a preservative. The syringes were capped and stored in ice water. Analyses were completed within two to four hours. The subjects breathed through a Douglas valve into a recording spirometer. For determination of oxygen consumption the expired air was diverted into a Douglas bag. Gas mixtures other than air were supplied to the inspiratory side of the system from large Douglas bags. These mixtures consisted of 15% O_2 in N_2, tank oxygen (99.5%), and 5% CO_2, 21% O_2 in N_2. Resting measurements were made with the subject seated. Ventilation was also measured with the subject standing, just before exercise. Exercise was carried out in an airconditioned room at 25° C. on a treadmill at a speed of 5.1 miles per hour on a 3.5% grade. For most of the subjects this exercise rate was fairly severe. Oxygen consumption, measured in eight subjects about five minutes after beginning exercise, was $2,290 \pm 90$ cc./min. (range: $1,760 - 2,610$). Ventilation was recorded continuously and is expressed as ventilation at room temperature ($25 \pm 1°$ C.).[3] Arterial blood samples were taken at points during the breathing of various gas mixtures, at rest and exercise, when it became evident from the respiratory tracing that the ventilation rate had been nearly steady for at least 30 seconds. The subjects were studied on two separate days, one of which was devoted to the effects of the carbon dioxide mixture, and the other to the effects of the various oxygen mixtures.

Respiratory gas analyses were done with the Haldane apparatus or with a Pauling oxygen meter. Arterial blood oxygen content and per cent oxygen saturation were determined by a spectrophotometric method (1). The pH of whole blood was measured with a Cambridge Model R pH meter equipped with an inclosed glass electrode. Measurements were made at room temperature, usually 25–26° C., and the result was corrected to 37° C. by Rosenthal's factor (2). This temperature was arbitrarily chosen for all pH measurements on arterial blood. pH determinations were made in duplicate in nearly every case, and duplicate determinations agreed to within 0.02 pH units or less. The stability of the instrument was checked with a pH 4.00 acid phthalate buffer or a pH 7.01 phosphate buffer between each reading. The carbon dioxide content of whole blood was determined by the method of Van Slyke and Neill (3), and the plasma carbon dioxide content was estimated from this value and from the pH and hemoglobin by the line chart of Van Slyke and Sendroy (4). From the pH and CO_2 content of arterial plasma at 37° C., the CO_2 tension of arterial blood was calculated by the Henderson-Hasselbalch equation. For the calculation of CO_2 tension at 37° C., pK′ was taken to be 6.11, as found by Dill, Daly and Forbes (5). Where the method was evaluated, as described below, by equilibration of blood in a tonometer at 38° C., a pK′ value of 6.10 was used, as determined for this temperature by Hastings, Sendroy, and Van Slyke (6).

The determination of CO_2 tension by this indirect means

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[3] Range of barometric pressure: 747–757 mm. Hg.

depends upon several measurements and calculations, of which pH determination and temperature correction seem most liable to error. The validity of the procedure was therefore tested by equilibrating blood samples in tonometers with a variety of CO_2 tensions at 38° C. The actual CO_2 tension or "direct tension" was determined by Haldane analysis of the gas mixture in the tonometer. The blood was then removed from the tonometer, cooled to room temperature, and determination of "indirect CO_2 tension" carried out as described above, except that pH's were corrected to 38° C. In this way, 23 comparisons were made between direct and indirect CO_2 tensions. The direct tensions ranged from 19.5 to 58.1 mm. Hg, and the corrected direct pH's from 7.59 to 7.13. The per cent oxygen saturation of these bloods was between 70 and 100% in 13 instances, and between 25 and 70% in the remainder. The mean indirect CO_2 tension exceeded the direct by 1.62 mm. Hg, with a standard deviation of 2.03, and a standard error of 0.42. This difference, though small, was significant ($P < .01$). In addition, a comparison of indirect arterial CO_2 tension with alveolar CO_2 tension was made in 17 instances. The alveolar airs were collected during rest and exercise with the variations in technique for these conditions described by Dill and colleagues (7, 8), and also during hyperventilation on air and various CO_2 mixtures. Alveolar airs do not provide a satisfactorily exact check on arterial CO_2 tensions, but the comparison was thought to be required by the possibility that changes in the blood during strenuous exercise might seriously invalidate the corrections made in the indirect measurement of CO_2 tension. The alveolar tensions ranged from 17.9 to 76.7 mm. Hg, and the blood pH's from 7.59 to 7.15. The mean indirect arterial CO_2 tension exceeded the alveolar tension by 1.6 mm. Hg, with a standard deviation of 3.7. The difference was not significant. These agreements seem satisfactory. It is not clear why the indirect CO_2 tension is significantly greater than the direct. It seems most probable that the error lies in measurement of blood pH. The difference could be caused by a mean error of approximately .02 units below the true value. The deviations between direct and indirect CO_2 tensions were not more marked at extreme pH ranges than at more ordinary values. This is of importance because Rosenthal's temperature coefficient for whole blood was established for a pH range of 7.25 to 7.45, while the temperature coefficient of serum has been found to vary considerably at extreme pH ranges (6.6 and 7.8) (5). The present results indicate that Rosenthal's coefficient for human whole blood can satisfactorily be extended over the pH ranges encountered in this study.

RESULTS

The data on each subject relating respiratory volume to arterial blood measurements at rest and exercise and during the breathing of various gas mixtures are presented in Tables I and II.

Resting arterial blood

The mean values for resting arterial blood are presented in Table III. Of the 11 subjects, arterial blood was obtained from one on only a single occasion, and from two subjects on three occasions. In the remaining eight, samples were taken during two separate trials. In determining mean values each subject was given equal weight. The arterial oxygen saturation is within the general range of recent reports by Comroe and Walker (97.5%) (9), Wood and Geraci (97.9%) (10), and Drabkin and Schmidt (98.6%) (11). The pH is somewhat lower than that found for arterial blood by Dill, Edwards, and Consolazio (12) (7.40) and by Dill and associates (7.39) (13), but higher than that reported by Barr, Himwich, and Green (7.30–7.36) (14). The CO_2 tension is slightly higher than that reported by Dill and colleagues (41.0, 40.9) (12, 13), higher than that of Lilienthal and coworkers, by Riley's direct method (37.8) (15), close to that of Galdston and Wollack (42 direct, 44 indirect) (16), and less than that of Barr and colleagues (44–45) (14). As noted above, the mean CO_2 tension is probably about 2 mm. Hg too high, and the pH .02 units too low. In addition, the values are probably affected by the small dead space of the respiratory apparatus, and are almost certainly affected by the fact that the subjects were not in a basal state, but came to the experiment directly from their daily activities. Under these conditions the variability in pH and CO_2 tension among normal subjects was quite striking.

Arterial blood changes during exercise

The present observations are not directly comparable to many of those reported in the literature for the reason that the exercise period was too short in most cases for the subject to reach a steady state. In addition, the subjects were untrained, and the exercise accordingly varied in its relative severity. For some subjects it was severe enough to preclude reaching a steady state.

The arterial blood oxygen capacity rose in all subjects, the mean increase being 6%. This is within the general range previously reported (8, 14, 17–19). The increase had reached nearly its full value after three minutes of exercise. The per cent oxygen saturation gradually declined dur-

TABLE I

Effect of 5% CO_2 during exercise

Subject, Surface area	State	Inspired mixture	Time on mixture	Ventilation	% Arterial O_2 saturation	Arterial serum CO_2	Arterial pH	Arterial CO_2 tension
(*sq. m.*)				(*l./min., 25° C.*)		(*vol. %*)		(*mm. Hg*)
R.	Rest	Air		6.1	95.8	53.2	7.41	38
1.93		5% CO_2	2:00	9.6	98.4	57.1	7.34	47
	Exercise	Air	3:25	51.4	95.9	42.9	7.26	37
		5% CO_2	1:38	81.8	95.8	46.2	7.19	53
		Air	1:50	78.6	94.2	34.5	7.20	39
R.	Rest	Air		6.4	97.5	53.5	7.38	41
1.86		5% CO_2	1:50	22.6	99.6	56.2	7.32	49
	Exercise	Air	2:45	71.1	98.8	40.7	7.29	38
		5% CO_2	1:10	122.0	98.2	47.7	7.13	62
		Air	0:54	84.0	97.8	34.5	7.21	38
M.	Rest	Air		9.7	99.3	51.0	7.38	39
1.90		5% CO_2	2:00	11.5	98.7	54.4	7.29	50
	Exercise	Air	3:50	58.0	97.1	43.3	7.20	49
		5% CO_2	1:27	87.0	97.4	49.6	7.13	65
		Air	1:00	67.0	97.5	40.3	7.23	42
K.	Rest	Air		6.5	98.2	58.1	7.38	44
1.88		5% CO_2	2:00	17.5	99.7	60.1	7.32	52
	Exercise	Air	4:00	40.3	97.8	48.5	7.29	45
		5% CO_2	2:00	72.7	99.1	53.1	7.19	61
		Air	2:10	51.1	97.8	45.1	7.27	43
D.	Rest	Air		15.9	99.4	52.6	7.50	31
2.12		5% CO_2	1:50	16.8	99.2	59.5	7.34	49
	Exercise	Air	3:40	49.4	95.1	51.9	7.29	48
		5% CO_2	1:00	66.0	96.8	55.9	7.14	71
		Air	2:10	65.0	94.0	44.6	7.21	49
L.	Rest	Air		8.3	98.4	56.7	7.35	46
1.95		5% CO_2	2:00	15.2	98.7	61.3	7.25	62
	Exercise	Air	3:45	42.0	97.0	51.5	7.20	58
		5% CO_2	1:15	57.0	97.5	53.2	7.10	73
		Air	1:50	59.7	95.6	42.8	7.13	56
C.	Rest	Air		6.3	98.4	56.4	7.36	45
1.92		5% CO_2	1:50	10.7	98.8	60.6	7.29	56
	Exercise	Air	3:30	44.3	98.3	53.2	7.31	47
		5% CO_2	1:45	81.5	98.5	57.6	7.22	62
		Air	1:00	51.0	97.8	50.0	7.32	43
	Rest	Air		7.6	97.4	55.7	7.35	45
		5% CO_2	2:45	17.6	99.2	59.9	7.31	53
G.	Rest	Air		9.1	98.9	60.7	7.40	44
2.04		5% CO_2	1:45	14.0	99.1	63.1	7.33	53
	Exercise	Air	3:15	47.0	96.9	52.7	7.28	50
		5% CO_2	1:00	58.0	97.5	58.7	7.19	67
		Air	1:10	52.4	96.1	53.0	7.27	51
S.	Rest	Air		7.1	97.8	56.4	7.37	44
1.89		5% CO_2	1:50	22.2	98.9	59.2	7.32	51
	Exercise	Air	3:27	37.4	97.3	52.7	7.34	43
		5% CO_2	1:20	72.5	97.6	59.9	7.20	67
		Air	1:03	49.9	97.4	51.4	7.27	49
T.	Rest	Air		6.9	96.7	57.5	7.36	46
2.04		5% CO_2	1:45	11.8	98.9	61.8	7.29	57
	Exercise	Air	3:15	30.0	96.8	58.5	7.29	54
		5% CO_2	1:02	27.3	97.2	64.0	7.19	73
		Air	1:50	31.0	98.1	57.8	7.29	53
	Rest	Air		5.9	97.7	58.6	7.36	47
	Exercise	Air	4:10	20.5	93.1	57.8	7.23	61
		5% CO_2	2:05	34.8	97.3	60.6	7.15	75
		Air	2:46	36.0	95.8	53.6	7.22	58

TABLE II

Effect of 15% O_2 and tank O_2 during exercise

Subject	State	Inspired mixture	Time on mixture	Ventilation	% Arterial O_2 saturation	Arterial serum CO_2	Arterial pH	Arterial CO_2 tension
				(*l./min., 25° C.*)		(*vol. %*)		(*mm. Hg*)
H.	Rest	Air		8.9	99.4	51.6	7.38	39
	Exercise	Air	2:50	66.6	97.7	43.5	7.28	41
		15% O_2	0:54	94.8	86.9	37.2	7.27	36
		Air	1:25	87.4	95.4	33.1	7.15	41
	*Exercise	Air	8:00	84				
		100% O_2	1:15	70				
R.	Rest	Air		7.6	97.6	55.0	7.37	43
	Exercise	Air	3:00	72.0	98.0	43.4	7.23	46
		15% O_2	1:00	97.0	86.9	38.1	7.21	42
		Air	1:10	72.0	95.4	36.5	7.16	45
		100% O_2	1:30	63.0	100.0	36.4	7.18	43
M.	Rest	Air		12.7	99.2	52.1	7.44	35
	Exercise	Air	2:40	65.0	98.0	44.4	7.32	38
		15% O_2	1:24	84.0	89.4	39.4	7.31	35
		Air	1:10	73.0	97.3	40.7	7.25	41
		100% O_2	1:10	49.0	100.0	38.2	7.20	43
K.	Rest	Air		8.3	99.4	54.6	7.32	47
	Exercise	Air	1:36	69.1	99.1	48.8	7.27	47
		15% O_2	1:15	63.0	86.7	41.8	7.25	42
		100% O_2	1:24	64.0	100.0	40.0	7.19	46
		Air	1:15	70.6	94.5	38.4	7.23	40
D.	Rest	Air		13.9	98.7	51.3	7.42	36
	Exercise	Air	3:36	40.7	96.2	50.5	7.23	53
		15% O_2	1:20	61.2	78.0	46.5	7.22	54
		Air	1:15	53.5	95.8	43.7	7.17	52
		100% O_2	1:27	49.9	100.0	44.5	7.14	57
L.	Rest	Air		6.6	98.3	57.9	7.33	49
	Exercise	Air	3:36	40.0	97.1	52.6	7.26	52
		15% O_2	1:46	40.0	88.7	49.8	7.24	51
		Air	1:57	48.0	96.0	49.2	7.21	54
		100% O_2	1:46	43.0	100.0	48.6	7.15	61
		Air	1:45	54.0	95.0	46.9	7.14	60
C.	Rest	Air		7.8	98.0	56.3	7.29	52
	Exercise	Air	2:36	40.6	97.9	50.9	7.29	47
		15% O_2	1:36	56.5	90.4	47.9	7.26	47
		100% O_2	2:00	38.8	100.0	54.7	7.30	49
		Air	1:54	51.0	97.3	49.4	7.32	43
B.	Rest	Air		7.4	99.7	54.0	7.32	47
	Exercise	Air	3:10	37.2	96.6	50.8	7.24	52
		15% O_2	1:36	53.0	88.4	49.2	7.26	48
		100% O_2	2:25	40.6	100.0	49.4	7.24	51
		Air	2:05	47.0	96.5	48.8	7.29	45
G.	Rest	Air		9.6	99.0	57.4	7.38	44
	Exercise	Air	3:51	37.6	97.8	54.0	7.34	45
		15% O_2	1:40	46.0	89.3	53.4	7.36	42
		Air	2:18	40.5	97.1	52.6	7.31	47
		100% O_2	1:39	35.0	100.0	53.1	7.29	49
		Air	1:57	40.9	96.7	53.2	7.31	47
S.	Rest	Air		8.8	95.3	59.3	7.33	50
	Exercise	Air	2:20	36.6	95.3	55.5	7.20	62
		15% O_2	2:10	50.9	84.4	49.8	7.23	52
		100% O_2	2:40	32.4	100.0	54.1	7.18	63
		Air	2:36	29.7	93.0	56.7	7.20	63
T.	Rest	Air		9.9	98.1	58.6	7.35	47
	Exercise	Air	2:36	31.4	97.6	55.2	7.28	52
		15% O_2	1:50	31.4	73.6	54.3	7.23	57
		100% O_2	2:00	36.3	100.0	51.8	7.24	53
		Air	1:20	36.6	95.3	50.0	7.20	56

* Separate study.

TABLE III

Mean arterial blood values of 11 subjects at rest and during exercise

State	Ventilation	Blood CO_2 content	Serum CO_2 content	pH	CO_2 tension	O_2 capacity	% O_2 saturation
	(*l./min., 25° C.*)	(*vol. %*)	(*vol. %*)		(*mm. Hg*)	(*vol. %*)	
Rest	8.7±0.7	46.3±0.7	55.4±0.8	7.37±.01	43.3±1.5	19.51±0.32	98.3
Exercise 3'11"*	47.1±4.0	41.8±1.2	49.9±1.5	7.27±.01	48.2±1.6	20.52±0.37	97.1
Exercise 8'20"*	57.2±4.6	38.1±1.7	45.4±2.1	7.22±.02	47.6±2.1	20.67±0.33	96.1

* Average time. (Ranges: 1'36" to 4'30" and 5'45" to 11'30".) Indicated range following mean values denotes standard error.

ing the exercise period, falling by an average of 2.2%. A similar fall has been reported by Asmussen and Nielsen (20). The arterial oxygen tension has been found to fall during exercise (15). In addition, the pH fall in our subjects would have the effect of shifting the oxyhemoglobin dissociation curve so that the per cent saturation would be less for a given oxygen tension.

For the group as a whole there was a considerable fall in pH and in serum CO_2 content. A large change had occurred by the time of the first sample, and the decline was continued in the second sample. Decreases of arterial pH and CO_2 content during exercise are a reflection of the accumulation of lactic acid (8, 21), and changes of this magnitude indicate that the rate of exercise was severe for the group as a whole. With subjects in better training, exercise rates of this degree or much higher may be maintained without the accumulation of large amounts of lactic acid and without a decline in pH (8, 22). However, with exercise which is severe for the subject, fall in arterial pH and serum CO_2 content of this general magnitude have been reported (14, 21, 23). Our experience is similar to Barr's and Himwich's (23) in the finding that resting pH and CO_2 content may not be restored for many minutes after exercise.

The mean arterial CO_2 tension was slightly increased during exercise. Because of the great individual variation, the difference between the resting and exercising levels is not significant for the group as a whole. Nielsen (22) reported that the CO_2 tension falls during exercise and that the fall is greater with increasing severity of exercise. His observations were made during the steady state of exercise on two highly trained subjects, and the arterial CO_2 tension was calculated from the composition of expired air and the respiratory dead space. Data on this subject are relatively few. The observations of Barr and associates (14), and of Galdston and Wollack (16) showing a fall or no substantial change in arterial CO_2 tension were made on arterial blood drawn shortly after exercise was completed. Lilienthal and colleagues (15), measured CO_2 tension by Riley's method in arterial blood drawn during exercise from six subjects. The sampling time was comparable to that of the present study, but, judging from the oxygen consumption, the severity of exercise was somewhat less. They found an average increase of approximately 3 mm. Hg in arterial CO_2 tension during work. Dill and associates have investigated the arterial CO_2 tension during exercise as measured by "inspiratory" alveolar air. There was considerable individual variation, but the average tension, after a steady state was reached, was not far from the resting value (7, 8). Four trained subjects at graded work rates showed a moderate increase in CO_2 tension with lighter work rates and a gradual decline toward the resting level as the rate was increased (13). Briggs (24) studied subjects under a very large work load. In many of the best performers, there was a high CO_2 content in the expired air (up to 8% in two subjects), indicating a very high arterial CO_2 tension. In short, the available evidence indicates that the arterial CO_2 tension during exercise may rise, fall, or remain nearly unchanged depending upon the individual subject, the work rate, and the duration of work. In four cases in the present study the CO_2 tension within one minute after exercise was found to be substantially the same as the original resting value.

The course of ventilation during exercise

At the start of exercise, as found by Krogh and Lindhard (25), there is an immediate increase in ventilation. With our subjects, this increased ven-

tilatory rate was maintained with relatively minor fluctuations, particularly in the first four to five breaths, for roughly a minute. A rather sharp further increase in ventilation then occurred. This sharp "secondary rise" in ventilation was present consistently except in one subject, T., in whom it appeared in only one of three trials. The secondary rise usually took place within a period of one to three breaths. The time at which the secondary rise occurred varied from 30 to 67 seconds after the onset of exercise, with an average time of 52 seconds. The mean increase at this point was 10.4 ± 1.6 l./min. The ventilation thereafter rose much more gradually and smoothly, reaching a steady state in some cases. This secondary rise seems not to have been commented upon before, although close observations of breathing during early exercise have been made by Krogh and Lindhard (25) and by Asmussen and Nielsen (26). Their subjects were in good physical condition. Continuous recordings of ventilation throughout the course of exercise have been made by Rahn and Otis (27) and by Bruce and coworkers (28), without the finding of a sharp break in ventilation. In these studies the subjects were untrained, but the work rate was quite mild.

It seemed possible that the secondary rise might reflect the beginning of arterial blood changes resulting from exercise. This possibility was examined in two further subjects who showed a well-marked secondary rise on moderate exercise (corresponding to an O_2 consumption of about 1,400 cc./min.). The results are presented in Table IV. It is apparent that there was a rapid fall in pH and a rise in arterial CO_2 tension during early exercise. The total extent of these changes, as measured by the effect on ventilation of breathing CO_2 at rest, could account roughly for the magnitude of the secondary rise. However, the ventilation rise is sharp, while the blood chemical changes are more gradual. This difference may reflect a lag between arterial blood changes and the ventilatory response. It seems possible only to conclude that chemical changes may occur quite early in the arterial blood of untrained subjects even during moderate exercise, and that these changes may be responsible for the secondary rise in ventilation.

On stopping exercise, there was usually a sharp decrease in the ventilation rate, as noted by Asmussen and Nielsen (26). The magnitude of this decrease varied greatly. The mean fall was 8.8 ± 2.6 l./min. Because of individual variation, there

TABLE IV

Ventilation and arterial blood changes during early exercise and while breathing CO_2-enriched air at rest

Subject	Sampling time after starting exercise	Comment	Ventilation	Serum CO_2	pH at 37° C.	CO_2 tension
			(*l./min., 25° C.*)	(*vol. %*)		(*mm. Hg*)
R. M.	Rest		8.8	53.6	7.37	41
	32″–41″		12.7*	55.5	7.34	45
		Secondary vent. rise to 19.8 l./min. begins at 63 sec.				
	71″–82″		19.8	55.8	7.28	52
	3′23″–3′33″		36.5	50.9	7.21	55
	Rest	28 min. after exercise	8.1	49.0	7.32	42
	Rest	Breathing 3–4% CO_2 for 4 min.	15.4	53.7	7.29	49
E. S.	Rest		8.5	56.0	7.38	43
	16″–26″		20.7*	55.5	7.34	46
	45″–55″			56.6	7.32	49
		Secondary vent. rise to 32.5 l./min. begins at 52 sec.				
	2′49″–3′04″		43.6	53.1	7.27	51
	Rest	22 min. after exercise	9.7	51.5	7.34	43
	Rest	Breathing 4–5% CO_2 for 2½ min.	25.2	56.5	7.28	53

* Average after first four to five respirations (about 15 seconds) until secondary rise in ventilation. Treadmill exercise at 4 mph and a 4% grade, subjects in poor training. A sharp "secondary rise" in ventilation is associated with an increase in CO_2 tension. Subjects show normal response to breathing CO_2 at rest.

was no significant difference between the magnitude of the immediate increase at the beginning of exercise and the decrease at the end.

The effect of breathing 5% CO_2

In comparing the effect on ventilation of breathing 5% CO_2 at rest and during exercise, the difficulty arose that a number of our subjects were unable to continue exercise with the gas mixture long enough to reach a steady state. Accordingly, the mixture was given until it was evident from the respiratory tracing that the ventilation was no longer changing rapidly, and arterial samples were then taken. For comparison, the same procedure was adopted in the resting state. The average time on CO_2 at rest was two minutes and during exercise one and one-half minutes. The average time needed to reach a steady state on 5% CO_2 at rest probably exceeds five minutes and in individual cases may be much more (*22, 29,* 30). However, the breathing time used in the present study covers the period of rapid change in ventilation. In measuring the effect of CO_2 inhalation during exercise, it was necessary to select control levels for ventilation and arterial CO_2 tension. This was complicated by the fact that the subjects were not in a steady state, and ventilation was consequently increasing with time, apart from the effect of CO_2. As shown in Table I, during the same exercise period control measurements were made while the subject was breathing air, both before and after the administration of CO_2. The control value for ventilation while the subject was breathing CO_2 was estimated by assuming that the ventilation time curve would have followed a straight line between these two points if the subject had continued on air. The mean values for the two points on air, before and after giving CO_2, were 46.6 l./min. at three and one-half minutes of exercise, and 58.3 l./min. after seven and one-half minutes of total exercising time.[4] The mean estimated control ventilation during CO_2 breathing was 53.3 l./min., while the mean actual ventilation was 73.0 l./min. The estimated control ventilation is a little too low because the ventilation-time curve is not a straight line but is convex upward. The apparent effect of CO_2 is thereby magnified, but the effect is not great. In the second control period, measurements were made at an average of one and one-half minutes after changing from CO_2 to air. It might have been expected that measurements during this period would still be much influenced by the preceding CO_2 inhalation. However, the mean ventilation at this point (58.3 ± 4.4 l./min.) was not significantly different from that of the last control period in the oxygen experiments (56.0 ± 5.4 l./min.), which are described in the next section. In most of the latter, the preceding gas mixture was tank oxygen, which depresses ventilation instead of elevating it. It is concluded that ventilation in the second control period was not significantly influenced by the preceding gas mixture in either case. The mean CO_2 tension was approximately 47 mm. Hg in each control period. There was very little individual change between periods, and a simple average was taken for the CO_2 tension to be expected if the subject had continued on air. It appears that there is a rapid recovery from the effect of inhaling CO_2 during exercise.

TABLE V

Mean effect on 10 subjects of breathing 5% CO_2 at rest and during exercise

State	Ventilation (l./min., 25° C.)	CO_2 tension (mm. Hg)	Ventilation change/CO_2 tension change (l./min./mm. Hg)
Rest	8.3 ± 0.9	41.8 ± 1.5	
Rest, 5% CO_2	15.5 ± 1.4	52.6 ± 1.4	.80 ± .24
Exercise	53.3 ± 4.2*	46.9 ± 2.1*	
Exercise, 5% CO_2	73.0 ± 7.5	65.5 ± 2.0	1.05 ± .20

* Expected values, calculated from measurements during exercise before and after the administration of CO_2. See text.

The mean results for 10 subjects are presented in Table V. The mean ventilation response to an increase in arterial CO_2 tension during exercise exceeds that at rest, but the difference is not statistically significant ($0.3 < P < 0.4$). It was suggested by Nielsen (*22*) that the respiratory center becomes more sensitive to CO_2 during exercise, and that this change is an important factor in the regulation of breathing during exercise. Under the conditions of the present experiment, there is no evidence that exercise has sensitized the respiratory center to CO_2 in the sense that the ventilatory response to a given increment in CO_2 ten-

[4] The four minutes between these sampling times included one and one-half minutes each on CO_2 and air, with approximately 30 additional seconds after each period which were occupied in completing the blood sampling and observing the ventilation rate to insure that it was not changing rapidly.

sion becomes significantly greater. The same conclusion was reached by Grodins (31) from an examination of Nielsen's data. The magnitude of the response to 5% CO_2 at rest is somewhat lower than usual, probably because of the short exposure time. The mean pH fall as a result of breathing CO_2 was slightly greater during exercise than at rest. As a corollary to the first conclusion, there appears to be no evidence that exercise produces any marked respiratory sensitivity to a fall in arterial pH.

The effect of high and low oxygen concentrations

In 1920 Briggs (24) observed that breathing oxygen-enriched air during severe exercise decreased the ventilation rate. The effect was greater the more severe the exercise, and for a given exercise rate the effect varied inversely with physical fitness. The finding has been confirmed abundantly (20, 22, 27, 32, 33). Conversely, it is known that ventilation for a given work rate is greater at high altitudes (13, 34, 35) or while breathing low-oxygen mixtures (15, 27, 36, 37) than it is at normal oxygen tensions. In the present study a trial was made of the effect on ventilation of changing from air to 15% O_2 in N_2 and to tank oxygen.

The first control measurements were made after about three minutes of exercise. The subject was then switched to 15% oxygen, and thereafter to air or tank oxygen. In all cases there were two or three control periods on air, as indicated in Table II. The time on each mixture was roughly one and one-half minutes before measurements were made. The subjects did not know the order in which mixtures were given and the changes were made without their knowledge. The method of determining "expected" ventilation and per cent oxygen saturation from the control periods was the same as described in the preceding section. The mean results for 11 subjects are presented in Table VI. Fifteen % O_2 caused a significant increase in ventilation ($P < .05$) and tank oxygen a significant decrease ($P < .01$), with reference to the rate on air. On switching gas mixtures, the change in ventilation rate was extremely rapid. This was particularly true in passing to oxygen, when a marked slowing was often evident within 10 seconds, or four to five breaths. With the ventilation changes there were corresponding changes in arterial CO_2 tension and pH. That is, with the lower ventilation under tank oxygen the CO_2 rose and the pH fell, while reverse changes occurred with 15% O_2. These secondary changes must have limited the extent of the oxygen effect, since they would force ventilation in the opposite direction.

TABLE VI

Mean effect on 11 subjects of breathing 15% O_2 and tank O_2 during exercise

State	Ventilation	% Arterial O_2 saturation	Ventilation change
	(l./min., 25° C.)		*(l./min.)*
Exercise, air	52.1±4.8*	96.9*	
Exercise, 15% O_2	61.6±6.5	85.7	9.5±3.1†
Exercise, air	55.8±5.3*	96.1*	
Exercise, tank O_2	47.4±3.9	100.0	8.4±2.3‡

* Expected values, calculated from measurements during control periods. See text.
† $p < .02$.
‡ $p < .01$.

It is of some interest to note that the mean change of CO_2 tension was only about 3 mm. Hg on changing from air to either gas mixture, which was less than might have been anticipated from the change in ventilation. The short exposure time may not have permitted the development of a full change in CO_2 tension. In addition, it is possible that the different gas mixtures may have influenced the rate of bicarbonate breakdown and consequently of CO_2 excretion. From the rate of total blood CO_2 loss, it is apparent that lactic acid was being formed rapidly. The rate of production of lactic acid during work can be markedly slowed by high oxygen mixtures or accelerated by low oxygen (20, 36, 38, 39).

The effect on ventilation of changing arterial oxygen tension is far greater during exercise of this severity than at rest. The effect on ventilation of inhaling high and low oxygen concentrations at rest has been studied by Dripps and Comroe (40). They found a small, immediate drop of 3.1% in ventilation on passing from air to oxygen. On 12% oxygen, which produced a mean fall of 17% in arterial saturation, approximately corresponding to that of the present study, there was an average ventilation increase of 0.5 l./min.

The effect of changes in arterial pH

There has been some disagreement as to the role played by arterial pH in the regulation of breath-

TABLE VII

Arterial blood pH, ventilation, and CO_2 tension in the post-exercise period

Subject	State	Ventilation	Arterial blood pH	Arterial blood CO_2 tension
		(l./min., 25° C.)		*(mm. Hg)*
R. M.*	Rest	8.8	7.37	41
	post-exercise 28 min.	8.1	7.32	42
E. S.*	Rest	8.5	7.38	43
	post-exercise 22 min.	9.7	7.34	43
S.	Rest	7.1	7.37	44
	post-exercise 6 min.	8.4	7.33	45
P. S.	Rest	7.8	7.35	45
	post-exercise 18 min.	10.7	7.29	47

* Data repeated from Table IV.

ing during exercise. Barr (14, 41), Dill, Talbott and Edwards (8), and Nielsen (22) found little correlation between pH and ventilation. From studies on diabetic acidosis Kety and associates (42) have suggested that there is a threshold for respiratory response at about pH 7.2. Between this point and pH 7.0, ventilation increased as the hydrogen ion concentration rose. These investigators pointed out that the arterial CO_2 tension of their patients was simultaneously decreased. As emphasized by Gray (43) and Grodins (31) such a change could mask a stimulant effect which might otherwise appear at higher pH levels.

Some data on this point are available from the present study and are presented in Table VII. In the post-exercise period, ventilation and arterial CO_2 tension in these subjects have returned substantially to their former values, while the pH is still .04 to .06 units below the control level. The pH changes are not large, but it seems possible to conclude that small pH changes, produced in this way, do not evoke much respiratory response. No such data are available, from this study, for large pH changes. As pointed out above, the finding that exercise does not generally increase respiratory sensitivity to CO_2 also implies that it does not cause a marked increase in sensitivity to a fall in arterial blood pH, since the pH was decreased considerably both at rest and exercise by CO_2 inhalation.

Individual variation

Individual variation is conveniently examined by reference to Figure 1. There is a striking difference among subjects in response to various stimuli.

The most consistent response is the immediate increase in ventilation on beginning exercise (Figure 1B). Even so, this shows up to 100% variation among subjects, and its magnitude is not closely related to the final ventilation volume. In Figure 1C is presented the abrupt fall in ventila-

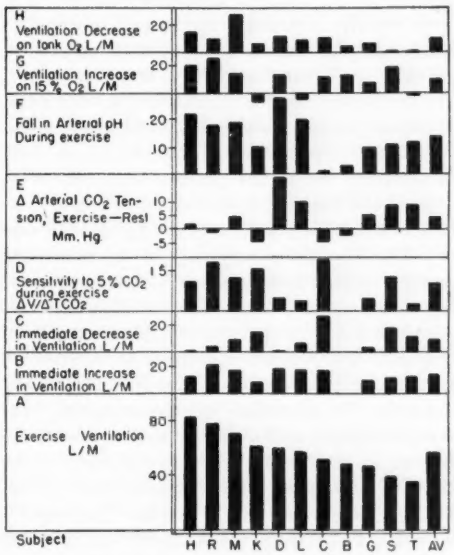

FIG. 1. VARIATION IN PERFORMANCE OF INDIVIDUAL SUBJECTS DURING EXERCISE

Columns at the extreme right represent the mean for each category. The values of categories A, B, C, E, and F are the averages of observations during two separate exercise periods, except for subject B, who had only one exercise period. A: ventilation during arterial blood sampling in the latter part of the exercise period. B: abrupt increase in ventilation on beginning exercise. C: abrupt decrease in ventilation on stopping exercise. D: increase in ventilation (l./min.) per mm. Hg rise in arterial CO_2 tension resulting from administration of 5% CO_2 during exercise. E: difference between arterial CO_2 tensions at exercise and rest. F: fall in arterial blood pH during exercise. G: increase in ventilation resulting from administration of 15% O_2 during exercise. H: decrease in ventilation resulting from administration of tank oxygen during exercise.

tion on stopping work. Here there is even a greater variation. In some subjects there is little or no immediate fall, while others show a fall greater than the original increase. The initial increase, because of its rapidity, has been considered neurogenic from the time of its description by Krogh and Lindhard, and the same etiologic reasoning is evidently applicable to the abrupt fall. Since the discovery by Harrison, Calhoun, and Harrison (44) of respiratory reflexes from passive limb motion, considerable attention has been devoted to this phase of respiratory control, most notably by Comroe and associates (45, 46), who have recently concluded that passive stretch reflexes can play only a minor part in respiratory regulation. Whether peripheral or central in origin, the present effect is of considerable magnitude. It is quite possible that the actual magnitude of this "neurogenic factor" during exercise is not fully measured by the abrupt changes in ventilation on starting and stopping exercise. A preliminary examination of this possibility has been made in subject H., who customarily shows an immediate increase in ventilation of about 12 l./min. on starting exercise and no immediate decrease on stopping, once a large ventilation volume has been reached. This subject was caused to exercise repeatedly for short periods with only brief recovery intervals. The immediate increase on passing from rest to exercise amounted in the first trial to 18 l./min., in the second, to 28 l./min., and in the third to 32 l./min., the last representing an immediate jump from 18 to 50 l./min. The possibility exists that the neurogenic factor may increase during exercise and become much larger than indicated by the average values for starting and stopping effects.

There is a very large range in sensitivity to arterial CO_2 tension, as shown by Figure 1D, representing ventilation increase per mm. of arterial CO_2 tension when 5% CO_2 is administered during exercise. Wide variation among subjects in response to carbon dioxide at rest has also been remarked (29, 30). Figure 1E presents the difference between arterial CO_2 tension during the latter part of exercise and the tension at rest. There is some correspondence between CO_2 sensitivity (Figure 1D) and the behavior of the arterial CO_2 tension during exercise. Where sensitivity is high, the CO_2 tension tends to fall or to rise only slightly during exercise. The large increase shown by D. is probably owing to anxiety and hyperventilation during the resting period, with a resultant subnormal CO_2 tension at rest. An exception is provided by S., who was sensitive to CO_2, but still showed a considerable increase in CO_2 tension during exercise. Evidently, the importance of CO_2 as a respiratory regulator during exercise is subject to considerable individual variation.

The reaction to 15% oxygen and tank oxygen is presented in Figures 1G and 1H. Once more, there was a considerable range of response. In several cases there was much difference in sensitivity to the two mixtures. The response to tank oxygen was a little more consistent than that to 15% oxygen.

DISCUSSION

In 1944 Comroe (47) reviewed the evidence dealing with respiratory regulation during exercise and concluded that a number of factors must act together. Subsequent work, including the present, has confirmed the conclusion. Efforts have been made to quantitate the role played by various of these factors. Most of this work has been done with well-trained subjects. It is generally believed that neurogenic stimuli play some part, although there is disagreement as to whether reflexes originating from the limbs themselves are important or not (46, 48, 49). It has been held, on the one hand, that neurogenic stimuli are of primary importance in light to moderate exercise (50), and, on the other, that they are of little importance. Carbon dioxide has been invoked as a major factor on the ground that exercise increases the responsiveness of the respiratory center to this stimulus (22), and the significance of carbon dioxide as a major factor has been denied on the ground that the alveolar carbon dioxide tension remains unchanged or falls during exercise (47). pH change has not been considered of first-line importance by most observers (8, 22, 41), but its possible importance has recently been asserted (31). Arterial oxygen tension has been dismissed as a regulatory factor because changes in arterial oxygen during exercise are slight, and change in arterial oxygen tension over a considerable range yields little respiratory response at rest (20). A rise in body temperature during exercise may stim-

ulate respiration, but this is slow to develop and probably would not be very effective in less than 10 minutes (51). Because it has often seemed difficult to account for the magnitude of ventilation during exercise, it has been recurrently postulated that an unidentified substance which acts as a respiratory stimulant is produced by working muscles.

In the present study, using untrained subjects, it is apparent that a number of stimuli were operative. Neurogenic factors, of whatever origin, played a significant part in initiating and maintaining ventilation. This stimulus may have been much larger than can be estimated from initial increases and terminal decreases in ventilation.

Arterial carbon dioxide tension appears to have been effective in increasing ventilation during exercise in at least some subjects. Since the respiratory center did not become highly sensitive to carbon dioxide during exercise, the effect as calculated after several minutes of exercise is not particularly large except perhaps in subject S., who was sensitive to carbon dioxide and had a substantial increase in arterial tension during exercise. In those subjects who have a rapid, early decrease in blood CO_2 content, presumably owing to lactic acid accumulation, a rising arterial CO_2 tension early in exercise may assist in increasing ventilation. As pointed out above, this may account for the "secondary rise" in ventilation early in exercise. In trained subjects this secondary rise has not been described, and trained subjects show little, if any, early increase in alveolar CO_2 tension (8, 22) corresponding to that found in the arterial blood of some of our untrained subjects.

The effect of changing the arterial oxygen tension is of considerable interest. The ventilation response is very rapid, and its magnitude is many times greater than at rest. This effect appears in exercise which is severe for the subject, and the effect becomes more pronounced as the severity increases (20, 24). The response is too immediate to make it likely to result from changes in blood concentration of some substance produced by anaerobically working muscles. It has been suggested by Asmussen and Nielsen (20) that an increase in oxygen tension could act by decreasing chemoreceptor sensitivity to such a substance. If so, one would need to postulate that the substance continues to be produced on oxygen at the same rate as on air, although lactic acid production is decreased by oxygen. This is because the results of these investigators show an immediate return of ventilation to the same steady rate as before when the subject is switched back to air. It seems a little simpler to assume that the chemoreceptor system becomes more sensitive to the arterial oxygen tension itself, or that the respiratory center becomes more sensitive to the "tone" which is known to exist at normal oxygen tensions in the resting state (40). In the latter case, the oxygen effect could be regarded as a variety of neurogenic stimulus which becomes more active the more severe the exercise. There is some evidence in animals for a respiratory drive from increased chemoreceptor tone, or increased responsiveness to the existing tone, during electrically induced work (49, 52). The development of this effect during exercise can make a significant contribution to the total ventilation. Normally, it can not provide for a very close regulation of ventilation because the effect does not vary greatly with a small change in oxygen tension. It is probably important in the close regulation of breathing during exercise at high altitudes and in patients with pulmonary disease who have a decreased arterial oxygen tension. For reasons outlined by Bohr (53) and Barcroft (54) and put to experimental trial by Lilienthal, Houston, and others (15, 35, 55), the arterial oxygen tension can fall markedly during exercise when it is already low at rest.

The large falls in arterial pH which occurred during exercise in some of our subjects would be expected to have some stimulant effect on respiration. Our experience, however, is similar to that of others in finding no close correlation between ventilation and pH in a given subject. As pointed out before, we find no evidence that the respiratory response to a fall in pH becomes very great during exercise.

Among the factors known to be operative in the present study, then, are a "neurogenic factor" which may increase during exercise, a changing arterial CO_2 tension, the development of what is provisionally regarded as an increased chemoreceptor tone to oxygen, and a considerable fall in pH. Recently, Gray (43) and Grodins (31) have approached the problem of multiple respiratory stimuli by assigning quantitative values to the stimulant effects of CO_2 tension, hydrogen ion con-

centration, and oxygen tension. If these values are accepted and the quantities measured, it is possible to draw conclusions as to the intensity and mode of action of additional stimuli which are required to account for the ventilation observed in a given situation. One of the most striking findings of the present study is the tremendous variability among untrained subjects in the response to different stimuli, as well as in the extent to which various stimuli develop during exercise. This suggests that any respiratory formula be applied only with reservations to a single subject or even to small groups of subjects, particularly when they are not in good physical training.

This great variability among normal subjects in poor physical condition is in contrast with the much more uniform performance of subjects in good training. It is probable that still greater deviations will occur in the course of disease. Present concepts of the relative importance of various respiratory stimuli in muscular exertion have been gained largely through the study of well-conditioned subjects. It does not appear likely that these concepts can be carried over intact to persons with acute or chronic disease.

SUMMARY

1. Observations have been made on ventilation and arterial blood changes in 11 untrained subjects during short periods of moderately severe treadmill exercise. Particular attention was paid to the ventilation changes at the start and conclusion of work, to the course of ventilation in early exercise, to the effect on ventilation of breathing gas mixtures having, respectively, an increased CO_2 content, a low oxygen, and a high oxygen content, and to associated changes in arterial CO_2 tension, pH and per cent oxygen saturation.

2. With untrained subjects there may be large changes during exercise in arterial CO_2 content, CO_2 tension, and pH, and these may begin to develop very early in exercise.

3. Exercise did not increase significantly the sensitivity of the respiratory center to arterial CO_2 tension, as measured by increase in ventilation per mm. Hg increase in CO_2 tension.

4. An attempt is made to estimate in semi-quantitative terms the role played by various respiratory stimuli in regulating ventilation during exercise. These stimuli are a "neurogenic" effect; an oxygen effect, which is provisionally regarded as an increase in sensitivity of the chemoreceptor system to oxygen tension; fall in blood pH; and arterial CO_2 tension. There is great variation among untrained subjects in the relative importance of these factors.

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THE EFFECT OF NOR-EPINEPHRINE UPON PULMONARY ARTERIOLAR RESISTANCE IN MAN [1]

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Nor-epinephrine is a sympathomimetic drug similar in structure to epinephrine, but lacking a methyl group on the nitrogen atom contained in the latter compound (1). Its levo-isomer is twice as active as the optically inactive preparation. Some investigators believe that l-nor-epinephrine is identical with sympathin E (1); others feel that it is more likely that nor-epinephrine is the precursor of epinephrine at adrenergic nerve endings (2, 3). Goldenberg and associates (1) found that in man l-nor-epinephrine increases total peripheral resistance, and in further contrast to epinephrine, does not increase the cardiac output. Goldenberg also (1) found a consistent elevation in pulmonary artery pressure during the infusion of l-nor-epinephrine in man. However, since he did not measure pulmonary venous pressure, one cannot determine from his study the primary site of action of l-nor-epinephrine in producing transient pulmonary hypertension. For these reasons it seemed desirable to investigate further the action of l-nor-epinephrine upon the pulmonary circulation.

The purpose of the study reported here is to determine how l-nor-epinephrine produces pulmonary arterial hypertension in man. In man the pulmonary venous pressure may not ordinarily be measured directly. However, Hellems and coworkers (4) have described a method of obtaining pulmonary "capillary" pressure, and have shown that in animals the pulmonary "capillary" pressure varies with the pulmonary venous pressure. If pulmonary artery pressure, pulmonary "capillary" pressure and cardiac output are determined simultaneously, the pulmonary arteriolar resistance may be calculated (5). If nor-epinephrine produces pulmonary hypertension by pulmonary arterial constriction, then a rise in pulmonary arteriolar resistance is to be expected during the pulmonary hypertension resulting from its exhibition.

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MATERIAL

Thirteen subjects were studied. Eleven had normal hearts as shown by physical examination, X-ray, and the electrocardiogram. One (E. B.) was convalescing from beriberi heart disease, with only residual tachycardia. One (C. F.) had a systolic murmur at the cardiac apex and a pulmonary "capillary" pressure tracing suggestive of incompetency of the mitral valve (6). C. F. had also moderate elevation of the mean pulmonary arterial pressure while in the resting state. As controls, six subjects with various types of cardio-pulmonary disease were studied.

METHOD

All subjects except E. B. were studied in the fasting condition, sedated by 0.1 or 0.2 gm. of seconal given two to two and a half hours prior to study. Cardiac catheterization was performed as described previously (7). A double lumen catheter was used, so that pulmonary "capillary" pressure and pulmonary arterial pressure could be measured simultaneously. Pulmonary "capillary" pressure was obtained by the method of Hellems and co-workers (4). The criteria of satisfactory pulmonary "capillary" pressures were (*1*) the nature of the pressure curve, showing "a" and "c" waves; (*2*) the peripheral location of the catheter tip in the lung; and (*3*) the securing of a specimen of blood saturated with oxygen from the wedged catheter tip. This last was not always possible, since in some instances, no blood could be obtained from the wedged catheter tip. Brachial artery pressure was obtained through an 18 gauge indwelling Cournand needle. Simultaneous electrocardiograms, ballistocardiograms, brachial arterial, pulmonary arterial, and pulmonary "capillary" pressures were recorded by means of a five channel optical oscillograph.

Cardiac outputs were determined by the direct Fick method as described previously (8). Duplicate samples of expired air were analyzed for CO_2 and O_2 and required to check within 0.03%. Duplicate blood samples, collected during the collection of expired air, were required to check within 0.2 vol.%.

Resting cardiac outputs were obtained after the catheter had been in place 15 to 30 minutes or more. After the

TABLE I

The effect of l-nor-epinephrine upon pulmonary artery and brachial artery pressure

Subject	Before nor-epinephrine		During nor-epinephrine		10 to 20 minutes after nor-epinephrine	
	Mean PA pressure	Mean BA pressure	Mean PA pressure	Mean BA pressure	Mean PA pressure	Mean BA pressure
	mm. Hg	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*
1. H. H. 39 W.M. Paresis	15	75	19	103	16	87
2. S. M. 40 C.M. Convalescent pneumonia	15	113	19	150	16	146
3. H. P. 48 W.M. CNS syphilis	16	130	17	146	14	125
4. G. Y. 46 W.M. Convalescent pneumonia	18	92	22	101	18	104
5. V. H. 27 W.M. Epilepsy	10	114	13	118	8	116
6. C. H. 49 C.F. Paresis	19	111	27	124	19	115
7. L. A. 43 W.M. Neuritis	13	113	18	127	13	112
8. E. J. 43 C.M. Leukemia	16	100	27	124	—	—
9. C. F. 50 C.M. Mitral incompetency	23	101	31	126	30	104
10. E. B. 28 W.F. Convalescent beriberi	17	110	24	133	—	114
11. J. D. 42 C.M. Bronchiectasis (minimal)	—	100	—	113	—	—
12. W. S. 26 C.M. Convalescent pneumonia	—	92	—	124	—	90

blood and gas samples for the resting cardiac output had been collected, the subjects were given 0.2 to 0.4 microgram of l-nor-epinephrine [3] per kilogram body weight per minute into the distal aperture of the catheter lying within the pulmonary artery. When a maximum effect upon the blood pressures had been obtained, usually within three to five minutes, blood and gas samples for a second cardiac output were collected. The infusion of l-nor-epinephrine was continued during the collection of samples. Pressure recordings were obtained immediately before and immediately after the collection of blood samples. In six cases a third cardiac output and set of pressure recordings were obtained 10 to 20 minutes after discontinuing the l-nor-epinephrine infusion. More than one resting cardiac output would have been desirable but was not obtained because of the danger of fatiguing the subject.

Six control subjects were studied by the above method. They received fluids comparable in volume and rate of infusion to those received by the subjects to whom nor-epinephrine was administered. Following resting measurements of cardiac output and pressures, 5% dextrose solution was infused into the pulmonary artery for a period of time roughly equivalent to that required for the infusion of l-nor-epinephrine. Then resting measurements of cardiac output and pressures were repeated.

Mean pulmonary arterial, brachial arterial and pulmonary "capillary" pressures were determined by planimetry. Measurements were made over two or more respiratory cycles. Pressures taken just before and just after each cardiac output were averaged in obtaining the final result. A point 10 cm. anterior from the back of the horizontal subject was taken as the zero point in pressure measurements.

Pulmonary arteriolar resistance was calculated by the formula:

$$R = \frac{PA - PC}{CO} \times 1{,}332.$$

Where R = arteriolar resistance in dynes/sec./cm.$^{-5}$
PA = mean pulmonary artery pressure
PC = mean pulmonary "capillary" pressure
CO = cardiac output in cc./sec.

Total peripheral resistance (TPR) was determined by the formula

$$TPR = \frac{BA}{CO} \times 1{,}332.$$

Where TPR = total peripheral resistance in dynes/sec./cm.$^{-5}$
BA = mean brachial artery pressure
CO = cardiac output in cc./sec.

Systemic peripheral resistance was determined according to the formula:

$$SPR = \frac{BA - RA}{CO} \times 1{,}332.$$

Where SPR = systemic peripheral resistance in dynes/sec./cm.$^{-5}$
BA = mean brachial arterial pressure
CO = cardiac output in cc./sec.
RA = mean right auricular pressure

RESULTS

Pulmonary arterial and brachial arterial pressure (*Table I*). In each of 10 subjects a definite rise in pulmonary artery pressure occurred. The mean rise in pulmonary artery pressure was 5 mm. Hg

[3] Supplied through the courtesy of Winthrop-Stearns Company, Buffalo, New York.

TABLE II

Statistical table of changes induced by l-nor-epinephrine

	No. cases	t—1% level	t—5% level	Observed t	p value *
1. Pulmonary artery mean pressure	10	3.250	—	5.7	<0.001
2. Brachial artery mean pressure	12	3.106	—	6.99	<0.001
3. Pulmonary "capillary" mean pressure	9	3.355	—	6.33	<0.001
4. Cardiac output	10	—	2.262	2.270	<0.05
5. Heart rate	12	3.106	—	4.3	<0.01
6. Total peripheral resistance	11	—	2.228	2.6	<0.05
7. Arteriovenous oxygen difference	11	—	2.228	1.85	<0.1
8. Stroke volume	11	—	2.228	1.94	<0.1
9. Correlation of rise in P. A. and P. C. mean pressure	9	r =0.76†	—	r =0.85†	<0.01

* From Fisher and Yates (10).
† Correlation coefficient (11).

or 33% over the resting value. Each of 12 subjects had a definite rise in brachial arterial pressure. The mean rise in brachial artery pressure was 20 mm. Hg or 19%. Ten to 20 minutes after cessation of nor-epinephrine administration, the pressure had fallen in all cases in the pulmonary artery, and in all cases save one in the brachial artery. However, 10 to 20 minutes was insufficient time for complete subsidence of nor-epinephrine effect in every case. The changes in both pulmonary artery and brachial artery pressures were statistically significant (9) at the $p < 0.001$ level (Table II).

Cardiac output (*Table III*). A significant rise (*i.e.*, 10% above the resting level [8]) in cardiac output was found in only one of the 11 cases. In this case the cardiac output rose still further after withdrawal of the nor-epinephrine; therefore the observed elevation was attributed to anxiety rather than the effect of the drug. A significant fall in output was observed in five subjects. In the five remaining subjects there was no significant change in cardiac output. Statistical analysis (Table II) revealed a significant decline in cardiac output when the group was considered as a whole ($p = .05$). The low cardiac index of subject C. F. is attributed to heavy sedation rather than to cardiac failure, since the subject had a normal arteriovenous oxygen difference of 3.9 vol.%. This subject was apparently unduly sensitive to barbiturates, and was roused with difficulty during the study, although he had received only 0.2 gm. of seconal. His oxygen consumption was quite low, being only 74.6 cc./sq. m./min.

Pulmonary "capillary" pressure and pulmonary arteriolar resistance (*Table IV*). There was a definite rise in pulmonary "capillary" pressure during the administration of nor-epinephrine in each instance. The mean rise was 6 mm. Hg or 64%, close to the mean rise in pulmonary artery pressure of 5 mm. Hg. Ten to 20 minutes after cessation of the nor-epinephrine infusion the pulmonary "capillary" pressures had fallen toward the original levels in all cases. The pulmonary arteriolar resistance showed no significant change in four subjects, a definite fall in three, and a definite rise in two subjects. The pulmonary arteriolar resistances showed much less tendency then pulmonary artery

TABLE III

The effect of l-nor-epinephrine upon cardiac output and heart rate

Subject	Before nor-epinephrine		During nor-epinephrine		After nor-epinephrine	
	Cardiac Index	Heart rate	Cardiac Index	Heart rate	Cardiac Index	Heart rate
	L/sq. m./min.	*per min.*	*L/sq. m./min.*	*per min.*	*L/sq. m./min.*	*per min.*
1. H. H. 39 W.M.	3.0	72	2.9	60	3.8	76
2. S. M. 40 C.M.	3.1	66	3.2	50	3.3	63
3. H. P. 48 W.M.	3.0	64	2.6	50	—	68
4. G. Y. 46 W.M.	2.3	82	3.3	65	3.8	88
5. V. H. 27 W.M.	3.2	65	3.3	55	3.9	64
6. C. H. 49 C.F.	3.4	82	3.4	78	—	86
7. L. A. 43 W.M.	4.3	92	3.5	60	4.7	88
8. E. J. 43 C.M.	3.3	65	3.4	70	—	80
9. C. F. 50 C.M.*	1.9	45	1.3	38	1.9	50
10. J. D. 42 C.M.	2.7	72	2.5	68	3.0†	68†
11. W. S. 26 C.M.	3.6	70	2.6	55	—	74
12. E. B. 28 W.F.	—	98	—	68	—	—

* Heavily sedated.
† Second control before drug administration.

TABLE IV

The effect of l-nor-epinephrine upon pulmonary "capillary" pressure and pulmonary arteriolar resistance

Subject	Before nor-epinephrine		During nor-epinephrine		After nor-epinephrine	
	P. C.	Pulm. res.	P. C.	Pulm. res.	P. C.	Pulm. res.
	mm. Hg	*dynes/sec./cm.$^{-5}$*	*mm. Hg*	*dynes/sec./cm.$^{-5}$*	*mm. Hg*	*dynes/sec./cm.$^{-5}$*
1. H. H. 39 W.M.	10	89	17	40	12	46
2. S. M. 40 C.M.	9	95	13	95	10	90
3. H. P. 48 W.M.	7	133	9	138	5	—
4. G. Y. 46 W.M.	11	131	17	65	12	69
5. V. H. 27 W.M.	6	66	11	37	7	27
6. C. H. 49 G.F.	9	151	15	163	6	—
7. L. A. 43 W.M.	9	41	12	75	6	63
8. E. J. 43 C.M.	7	114	17	122	—	—
9. C. F. 50 C.M.	14	202	22	326	16	333

mean pressures to return toward the original level in those cases where they were altered. The observed elevation in pulmonary "capillary" pressure was significant statistically ($p < 0.001$) (Table II).

Total peripheral resistance and heart rate (Tables III and V). A significant increase in total peripheral resistance (TPR) was found in nine of 11 subjects during the infusion of nor-epinephrine. There was no significant change in one subject, and a decline in resistance in another. The mean rise was 497 dynes/sec./cm.$^{-5}$ or 31%. The heart rate slowed in 11 of the 12 subjects. The one subject who did not show slowing (E. J.) demonstrated a further rise in heart rate after the cessation of nor-epinephrine, suggesting a masking of the drug effect by some extraneous factor. Both heart rate and peripheral resistance returned toward resting values 10 to 20 minutes after withdrawal of the l-nor-epinephrine. The observed bradycardia was statistically significant at the $p < 0.001$ level and the observed increase in total peripheral resistance was significant statistically at the $p < 0.05$ level (Table II).

TABLE V

The effect of l-nor-epinephrine upon total peripheral resistance

Subject	Before nor-epinephrine	During nor-epinephrine	After nor-epinephrine
	TPR	TPR	TPR
	dynes/sec./cm.$^{-5}$	*dynes/sec./cm.$^{-5}$*	*dynes/sec./cm.$^{-5}$*
1. H. H. 39 W.M.	1,223	1,698	1,119
2. S. M. 40 C.M.	1,727	2,222	2,160
3. H. P. 48 W.M.	1,918	2,474	—
4. G. Y. 46 W.M.	1,704	1,312	1,188
5. V. H. 27 W.M.	1,787	1,802	1,478
6. C. H. 49 C.F.	1,539	1,708	—
7. L. A. 43 W.M.	1,142	1,609	1,045
8. E. J. 43 C.M.	1,227	1,502	—
9. C. F. 50 C.M.	2,523	4,557	2,637
10. J. D. 42 C.M.	1,615	1,976	1,405*
11. W. S. 26 C.M.	1,170	2,187	—

* Duplicate observations before drug administration.

Right atrial pressure, right ventricular end diastolic pressure, and peripheral venous pressure. In each of three subjects in whom observations were made pressures within the right side of the heart rose during administration of the drug. In subject W. S. right atrial pressure was 29 mm. water in the resting state. During l-nor-epinephrine administration, the pressure rose to 82 mm. water. After the drug was discontinued, right auricular pressure fell to 38 mm. water. In subject E. B., right ventricular end diastolic pressure rose from 5 mm. Hg to 14 mm. Hg during l-nor-epinephrine infusion. Right atrial pressure also rose during the drug infusion but unfortunately the pressure was not measured at its peak and the rise was less striking than that in the right ventricular end diastolic pressure. In subject F. H. there was a striking rise in right atrial pressure during l-nor-epinephrine infusion (Figures 1 and 2). A peripheral venous pressure curve was obtained simultaneously with the right atrial and peripheral artery pressure curve in this subject (Figure 2). There was an approximately equal rise in pressure in the peripheral vein and right atrium approximating 10 mm. Hg in the right atrium and 11 mm. Hg in the peripheral vein. However, the peak pressure rise occurred earlier in the peripheral vein than in the right atrium (Figure 1).

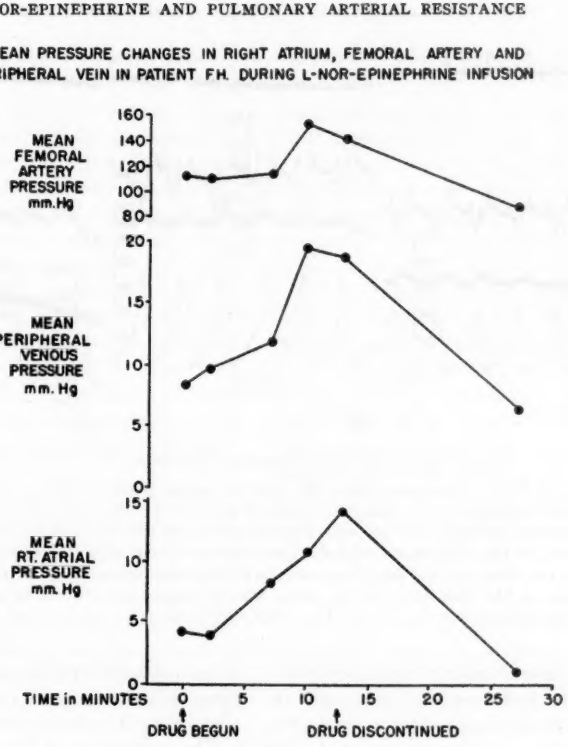

FIG. 1.

TABLE VI

Control subjects

	First resting study						Second resting study					
	BA mean	PA mean	PC mean	CO	TPR	Pulm. res.	BA mean	PA mean	PC mean	CO	TPR	Pulm. res.
				L/min.						*L/min.*		
1. R. J. 69 C.M. hypertension	—	16.8	6.8	3.6	—	223	—	16.4	8.8	3.4	—	181
2. M. L. 52 W.F. emphysema	87.3	20.8	5.8	3.6	1,961	337	90.3	21.5	5.1	3.5	2,092	380
3. M. S. 49 W.M. mitral stenosis	—	53.8	—	3.7	—	—	—	49.5	—	3.9	—	—
4. J. D. 48 C.M. bronchiectasis	99.6	—	12.8	4.9	1,615	—	93.4	—	11.8	5.3	1,405	—
5. D. H. 61 W.M. cor pulmonale	65.3	38.4	—	4.4	1,963	—	65.7	36.3	—	4.2	2,050	—
6. F. B. 18 C.F. sickle cell anemia	—	18.6	—	8.6	—	—	—	16.8	—	7.9	—	—

Mean pressures are expressed in mm. Hg.
Resistances are expressed in dynes/sec./cm^{-5}.
BA = brachial artery.
PA = pulmonary artery.
PC = pulmonary "capillary."
CO = cardiac output.

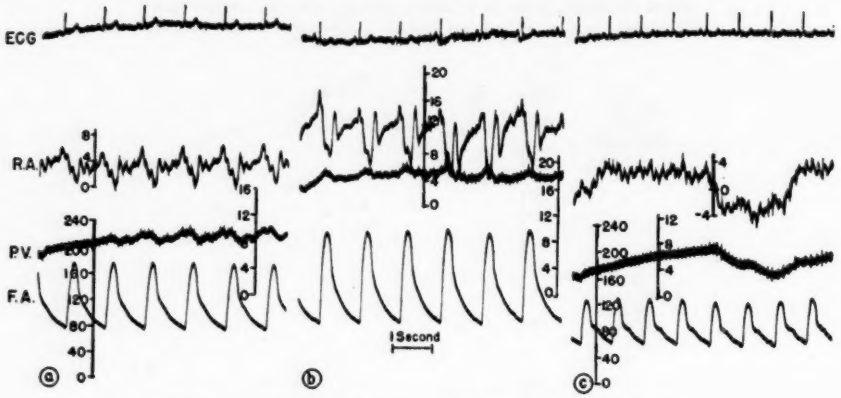

FIG. 2. EXCERPTED FROM PRESSURE TRACING OF PATIENT F. H.

Section (a) is before l-nor-epinephrine; (b) is at the peak response 13 minutes after beginning the infusion of l-nor-epinephrine; (c) shows the return of pressure to resting levels 14 minutes after cessation of l-nor-epinephrine infusion. In (a), mean femoral artery pressure is 111.6 mm. Hg; mean peripheral vein pressure is 8.4 mm. Hg; mean right atrial pressure is 4.2 mm. Hg. In (b), the mean femoral artery pressure has risen to 141.2 mm. Hg; mean peripheral vein pressure, to 18.7 mm. Hg; mean right atrial pressure, to 14.2 mm. Hg. In (c) mean femoral artery pressure has fallen to 88.4 mm. Hg; mean peripheral vein pressure, to 6.3 mm. Hg; mean right atrial pressure, to 1.1 mm. Hg.

In order to exclude changes in right atrial pressure as a cause of increased total peripheral resistance, the observed values for right atrial pressure should be subtracted from the brachial pressure in the formula. This could not be done because right atrial pressures were not routinely measured. In one subject (W. S.) in whom this could be done, the systemic peripheral resistance was 1143 dynes/sec./cm.$^{-5}$ before and 2082 dynes/sec./cm.$^{-5}$ during l-nor-epinephrine, showing an increase of 82%.

In the six control subjects (Table VI), the second resting cardiac output varied less than 10% from the resting measurement. The mean variation in pulmonary artery pressure was 1.7 mm. Hg. The observed variations in brachial artery pressure, pulmonary "capillary" pressure, total peripheral resistance, and pulmonary arteriolar resistance are seen in Table VI.

DISCUSSION

The hemodynamic changes observed during an infusion of l-nor-epinephrine consisted of increased pulmonary artery and brachial artery pressures, increased peripheral resistance, diminished cardiac output, and bradycardia, and confirm the observations of Goldenberg (1). The bradycardia was also observed by Judson and co-workers (12).

The mechanism of the increase in pressure in the pulmonary and peripheral arteries is not the same. There was a statistically significant elevation in total peripheral resistance during l-nor-epinephrine administration; on the other hand, in only two of the nine cases studied was there an increase in pulmonary arteriolar resistance. In one of these (C. F.) the pulmonary "capillary" pressure rose within the critical range of 20–25 mm. Hg, at which level pulmonary resistance increases as a protective mechanism to prevent pulmonary edema (5). Thus it is quite probable that the rise in resistance in case C. F. was not a direct effect of l-nor-epinephrine, but an effect secondary to the increase in pulmonary venous pressure. An increase in pulmonary "capillary" pressure was observed in all cases following l-nor-epinephrine, and the mean increase was nearly the same as the mean increase in pulmonary artery pressure. Therefore, the usual mechanism whereby l-nor-epinephrine produces pulmonary hypertension in man is through an increase in pulmonary "capillary" or venous pressure.

The rise in pulmonary artery pressure following l-nor-epinephrine infusion is due to a rise in left atrial pressure for the following reasons. First, there is no consistent rise in pulmonary arteriolar resistance after the drug, thereby excluding constriction of the pulmonary arterioles as the mechanism of pressure elevation. Second, there is a significant correlation between the rise in the pulmonary artery and pulmonary "capillary" mean pressures during l-nor-epinephrine infusion (Table II). Third, there is a definite rise in pulmonary "capillary" pressure following l-nor-epinephrine which implies a rise in pulmonary venous pressure and thus a rise in left atrial pressure. Fourth, there is a rise in right atrial pressure after the drug, which also implies a concomitant rise in left atrial pressure (13).

One may speculate further as to the mechanism of elevation of right atrial pressure following l-nor-epinephrine. Ranges and Bradley (14) and Bradley and Parker (15) in attempting to explain the rise in right atrial pressure after paredrinol and angiotonin, considered heart failure unlikely because there was no evidence of cardiac dilatation. They believed that an increase in venous tone, decreasing the total volume of the vascular system, might be responsible. Iglauer and Altschule (16) have shown that other sympathomimetic drugs—adrenaline and paredrine—increase the peripheral venous pressure. Ranges and Bradley also mentioned an increase in "cardiac tone" as the cause of the elevated right atrial pressure following angiotonin.

TABLE VII

Effect of l-nor-epinephrine upon A-V oxygen difference and upon stroke volume of the heart

Subject	Stroke volume (*cc.*)		A–V difference (*cc./100 cc. blood*)	
	Before l-nor-epinephrine	During l-nor-epinephrine	Before l-nor-epinephrine	During l-nor-epinephrine
1. H. H.	68	81	3.7	4.5
2. C. F.	71	58	3.9	5.5
3. S. M.	79	108	4.7	5.7
4. H. P.	85	94	4.5	5.2
5. G. Y.	53	95	4.7	4.7
6. V. H.	78	96	3.2	3.6
7. C. H.	70	74	4.0	3.2
8. L. A.	86	105	2.7	3.5
9. E. J.	100	94	5.3	4.4
10. J. D.	69	67	4.0	4.1
11. W. S.	89	82	3.0	4.6

Although there was an increase in the mean A–V oxygen difference of 12% in the subjects studied, there was also an increase in the mean stroke volume of 13% (Table VII). The changes in stroke volume and A–V oxygen difference were not significant statistically (Table II). Therefore it is believed that the decrease in cardiac output during l-nor-epinephrine infusion does not represent congestive heart failure (1). The striking increase in peripheral venous pressure observed in Subject F. H., reaching its peak earlier than the peak rise in right atrial pressure during the l-nor-epinephrine infusion, suggests that increased peripheral venous tone may indeed be responsible for the rise in intra-atrial pressure by decreasing the capacity of the vascular system. The change here is quite unlike that in congestive heart failure, where the gradient between pressure in peripheral veins and right atrium is diminished rather than increased (17), the pressure in the two sites often becoming almost equal as the pressure rises.

One must also consider the possibility that peripheral venous constriction is the mechanism primarily responsible for the hypertension induced by l-nor-epinephrine in the systemic circuit. Since a rise of 20 mm. Hg, the observed mean elevation in mean peripheral arterial pressure, was much greater than any of the measured increases in mean right auricular pressure (this relationship holding true in the three cases where both pressures were determined) and since the cardiac output is decreased or unchanged after l-nor-epinephrine, one is justified in stating that there is definite increase in systemic peripheral resistance during administration of the drug. In one case where systemic peripheral resistance was calculated, there was a striking increase of 82% during l-nor-epinephrine. Thus one may conclude that arterial or capillary constriction contributes to the observed increase in total peripheral resistance despite the rise in right atrial pressure.

SUMMARY AND CONCLUSIONS

The hemodynamic response of 13 subjects to the infusion of l-nor-epinephrine was studied. During l-nor-epinephrine infusion, the following changes occurred: a statistically significant mean rise in pulmonary artery pressure; a significant rise

in mean brachial artery pressure; a decrease in the cardiac output in five subjects, remaining unchanged in five. There was a statistically significant mean rise in pulmonary "capillary" pressure. There was a statistically significant increase in total peripheral resistance. There was no significant increase in pulmonary arteriolar resistance. There was a definite and marked rise in right atrial pressure in each of three subjects.

It is concluded that the pulmonary hypertension seen during the administration of l-nor-epinephrine is not due to constriction of the pulmonary arteries, but rather to an increase in pulmonary venous pressure; it is also concluded that l-nor-epinephrine produces constriction of peripheral arteries or capillaries as well as peripheral veins.

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BLOOD LEVELS OF ADENINE NUCLEOTIDES[1]

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INTRODUCTION

The metabolic significance of the adenine nucleotides (1, 2) and the reports of the therapeutic action of muscle adenylic acid and adenosine triphosphate in various pathological states (3–12) has stimulated interest in the possible mechanism of action in these compounds.

Recently a rapid reliable method was developed by one of us (13) for measuring total adenine, adenylic acid (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP). We have applied this method to the study of blood since it is the only human tissue which can be conveniently studied.

Before studies could be undertaken on pathologic bloods, we felt that normal bloods should be examined for total nucleotide content as well as for possible content of adenylic acid, adenosine diphosphate and adenosine triphosphate. That adenine nucleotides are present in blood has been known since 1914. The first completely adequate quantitative data are those of Buell in 1935 (14) who estimated total nucleotide nephelometrically after precipitating with uranium and then silver, from a neutralized trichloracetic acid filtrate. Apart from describing the procedure itself, Buell reported that the nucleotides are present principally in the red cells (indeed the value varies with the hematocrit); that the level for any one individual over a period of several months is fairly constant, and that the average value for males is about 11.5 mgm.% and for females 10.0 mgm.% (expressed as adenine).

Our interest, as indicated above, was not only in the total nucleotide, but also in the constitution of the nucleotide. We also felt that the Buell procedure for estimating nucleotides was much too cumbersome. We, therefore, concentrated our attention on the preparation of filtrates which could be assayed directly.

The procedure used in our work is a spectrophotometric assay on perchloric acid filtrates, using in modified form the enzyme techniques of Kalckar (15–17).

In addition to presenting observations on the concentration of the various nucleotides on a group of four normal subjects (three men and one woman) over a period of about eight months, data will be presented on the effects of intramuscular injections of muscle adenylic acid, adenosine triphosphate and yeast adenylic acid on the levels of the above compounds.

ANALYTICAL PROCEDURE

a) Estimation of total adenine

Total adenine nucleotide was assayed by drawing blood, initially under oxalate, and immediately deproteinizing 1 ml. with 4 volumes of cold 2% perchloric acid. The protein precipitate was washed with an additional volume of cold 2% perchloric acid. The combined supernatant solutions collected after centrifugation were adjusted to pH 7.0 and made to a final volume of 10 ml. One ml. of filtrate was added to a tube containing 3.5 ml. H_2O and 0.5 ml. M/10 phosphate buffer at pH 7.4. The resulting solution when read in the spectrophotometer shows an absorption maximum at 258 mμ, corresponding to the absorption maximum of adenine nucleotides in perchloric acid. Total nucleotide was calculated as adenine using muscle adenylic acid as a standard.

b) Estimation of AMP, ADP and ATP

Adenylic acid, adenosine diphosphate and adenosine triphosphate were assayed in the same filtrates according to the procedure described by Albaum and Lipshitz (13), in the following way: One ml. aliquots of the filtrate were placed in three separate tubes. Into the first tube 0.5 ml. 0.1 M malonate buffer pH 5.9, 0.03 ml. of adenylic acid deaminase (containing 15 μgm. protein N) and water to make a final volume of 5.0 ml. were pipetted and the change in density in the spectrophotometer at 265 mμ recorded at the end of 10 minutes. The second tube received buffer, water, 0.1 ml. $MgCl_2$ (10 mgm./ml.), 0.01 ml. myokinase (containing 4 μgm. protein N) and deaminase and again the change in density at 265 mμ was recorded at the end of 10 minutes. To the third tube was added buffer, water, $MgCl_2$, myokinase, 0.1 ml. 0.5 M glucose, 0.01 ml. hexokinase (containing 7 μgm. protein N) and the deaminase and once more the change in density determined. Any

[1] This work was supported by a grant to Brooklyn College from the Ernst Bischoff Company, Ivoryton, Conn.

change in tube 2 over that occurring in tube 1 is due to the presence of ADP. Since only 1 mole of AMP is formed from 2 moles of ADP, the difference between tubes 1 and 2 is multiplied by a factor of 2. The third tube records the change in density due to AMP, ADP and ATP. Since the concentration of AMP and ADP are now known (by reading the adenine due to these compounds from a standard curve derived from the change in density of known concentrations of these nucleotides) and the total adenine is known from tube 3, the difference between the AMP and ADP adenine and the total adenine gives the value for the ATP adenine.

The reactions discussed above are represented by the following equations:

$$(1)\ \text{AMP} \xrightarrow{\text{adenylic acid deaminase}} \text{Inosinic Acid}$$

$$(2)\ 2\text{ADP} \xrightarrow{\text{myokinase}} \text{ATP} + \text{AMP}$$

$$(3)\ \text{ATP} + \text{glucose} \xrightarrow{\text{hexokinase}} \text{ADP} + \text{glucose-6-phosphate}$$

Before experiments were undertaken on the distribution of the compounds, several preliminary runs were carried out to see whether the method as described was reliable, *i.e.*, could added nucleotide be recovered and measured? The results of one such experiment are shown in Table I. The total nucleotide accountable for as AMP, ADP and ATP in this sample of blood was 6.4 μgm. It is apparent that added ATP can be recovered indicating that the method is reliable.

Early in our studies on the concentration of the various nucleotide fractions in oxalated human blood, it was found that ATP content decreased significantly within the first hour, though not in the first five minutes. For this reason, in the early work which was done on oxalated blood, the samples were pipetted into perchloric acid within one minute of withdrawal. To avoid the inconvenience of this procedure, the stability of the nucleotide in heparinized blood was investigated. Table II shows the percentage of total purine found in the ATP, AMP and ADP fractions, respectively, in freshly drawn blood, and in the same blood samples after 90 minutes. The experiment was run at room temperature and at 37° C. in the presence of a mixture of potassium oxalate and ammonium oxalate, of which the oxalate concentration was 112 mgm. %, and in the presence of 5 mgm. % of heparin. It is clear that the ATP is very significantly split to ADP and AMP in oxalated blood, while it is quite stable in the heparinized blood.

Adenylic acid deaminase (Schmidt's deaminase) was

TABLE I

Recovery of ATP when added to perchloric acid filtrate of blood

1. Total AMP, ADP, and ATP found in blood sample = 6.4 μgm.
2. ATP added to blood sample = 8.2 μgm.
3. Total AMP, ADP, and ATP in blood and added ATP
   calculated (1 + 2) = 14.6 μgm.
   found = 14.3 μgm.

TABLE II

Effect of standing on adenine nucleotides in blood

Temperature		Time	% of total purine as		
			ATP	AMP	ADP
		min.			
Room	Oxalated blood	0	68	0	0
		90	15	28	25
	Heparinized blood	0	58	0	0
		90	59	0	4
37° C.	Oxalated blood	0	40	0	5
		90	12	20	7
	Heparinized blood	0	39	0	5
		90	44	0	0

prepared according to the method of Kalckar (15); myokinase, according to Colowick and Kalckar (18); hexokinase was prepared in partially pure form according to the method of Berger, Slein, Colowick and Cori (19) through step No. 3 (fractionation with ethanol at 0° C.) of the isolation procedure. The enzymes were kept in a deep freeze and were found to be stable over long periods of time.

Muscle adenylic acid used for injection was supplied by the Ernst Bischoff Co., Ivoryton, Conn. as My-B-Den (1 ml. ampules containing 20 mgm. as the sodium salt). In some experiments ampules containing 100 mgm./ml. were used. ATP was also obtained from the Ernst Bischoff Co. and was administered as a solution of the sodium salt.

EXPERIMENTAL

Total nucleotide content of blood

The total nucleotide content of the blood of the four subjects used in the experiments expressed as mgm. % adenine are shown in Figure 1 (closed circles). Several conclusions may be drawn from these data: *1.*) While there is some variability in total nucleotide, the levels remain fairly uniform over a period of about eight months. The variability is no greater than one finds in the levels of other blood constituents under normal conditions. *2.*) The levels obtained are similar to those obtained by Buell using the nephelometric procedure, so that we appear to be measuring the same thing that Buell measured.

Nucleotide present as AMP, ADP and ATP

That portion of the total nucleotide accountable for as AMP, ADP and ATP is also shown in Figure 1 (open circles). It is immediately clear that the level of these compounds shows considerably more variation than does the total nucleotide.

They may vary anywhere between 30 and 90% of the total nucleotide. Some of the early samples were collected before breakfast, the later ones after breakfast; and there appeared to be no consistent difference.

To further illustrate the difference in variability between the values for the total purine and those for the known nucleotide (AMP, ADP and ATP) we have calculated the mean, standard deviation and coefficient of variability for a large number of individual determinations (n = 78). For the total purine the following values were obtained: m = 10.5 mgm.%, the standard deviation 0.84, the coefficient of variability 8.0%. The corresponding values for the known nucleotide were m = 6.0 mgm.%, the standard deviation 1.58, and the coefficient of variability 26.5%.

Effect of injection of adenylic acid

The fact that the amount of nucleotide present as AMP, ADP and ATP is variable from day to day (see Figure 1), and that the total nucleotide remains fairly uniform, suggests that the former are individually in some kind of loose equilibrium with the total nucleotide. Can this equilibrium be shifted, *e.g.*, by the injection of adenylic acid? In these experiments 20 mgm. ampules of muscle adenylic acid as the sodium salt (My-B-Den) were used. Blood was taken from the antecubital vein, and immediately thereafter the contents of an adenylic acid ampule were injected into the other arm intramuscularly. Additional blood samples were withdrawn from the first arm 30, 60 and 90 minutes after the injection. The results of three typical runs are shown in Table III. It should be noted that most of the blood samples contained ADP and ATP, but no adenylic acid initially. After injection in all cases the ADP decreased and the ATP increased. The ATP level in two of the three cases began to fall at the end of 60 minutes and adenylic acid appeared. The total purine accountable for as AMP, ADP and ATP approached the value for the total nucleotide. In many cases the increases obtained in the known AMP, ADP and ATP fractions were not as marked as those shown, but they almost always occurred. In one case no increase was obtained in the first run, though in a subsequent run it did occur.[2]

[2] Since this paper went to press, the response of five additional normal subjects to the injection of muscle adenylic acid was tested. Four of the five showed a response similar to that in Table III. One subject showed no change in blood nucleotide. A later run on the same subject also gave a negative response.

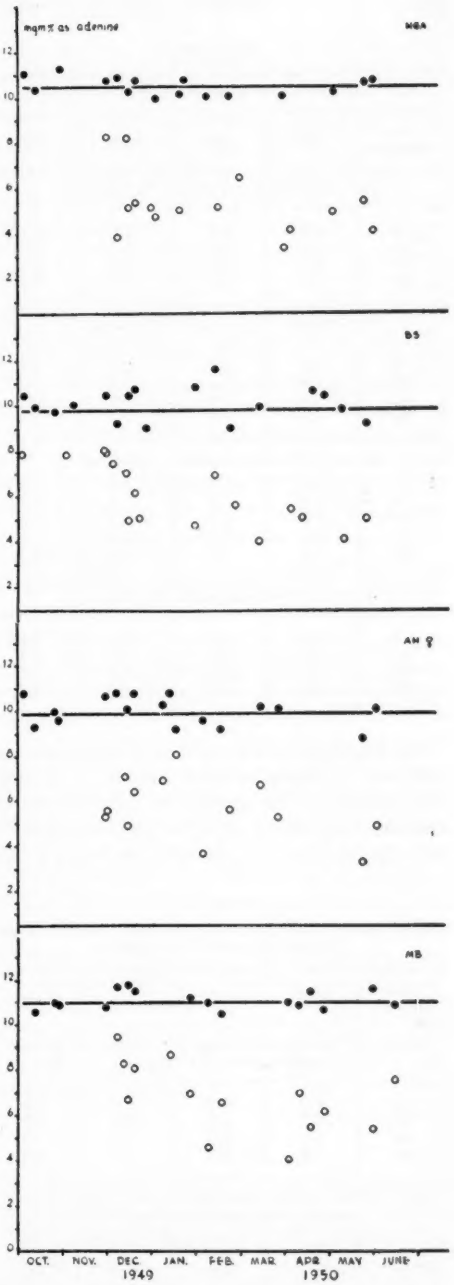

FIG. 1. TOTAL PURINE (CLOSED CIRCLES) AND NUCLEOTIDES PRESENT AS AMP, ADP AND ATP (OPEN CIRCLES) FOR A GROUP OF FOUR SUBJECTS (THREE MEN AND ONE WOMAN) OVER A PERIOD OF EIGHT MONTHS

TABLE III

Effect of intramuscular injection of 5-adenylic acid: 20 mgm. (My-B-Den) on blood nucleotides (AMP, ADP, ATP)

Time (*minutes*)	AMP	ADP	ATP	Total
AH 1/18/50—Total purine 9.2 mgm. %				
→0	0.0	6.5	1.7	8.2
→30	0.0	4.5	4.3	8.3
60	0.0	0.8	8.0	8.8
90	1.2	0.0	8.0	9.2
HA 1/20/50—Total purine 10.2 mgm. %				
→0	0.0	4.1	1.0	5.1
→30	0.0	3.4	4.4	7.8
60	0.0	0.0	10.0	10.0
90	2.4	0.0	7.1	9.5
MB 1/23/50—Total purine 11.2 mgm. %				
→0	0.0	3.7	3.3	7.0
→30	0.0	3.4	6.8	10.2
60	0.0	2.5	7.6	10.1
90	1.1	3.4	4.0	8.5
BS 3/13/50—Total purine 10.0 mgm. %				
→0	0.0	0.0	4.1	4.1
→30	0.0	0.0	7.7	7.7
60	0.0	0.0	9.1	9.1
90	0.0	0.0	7.7	7.7

The magnitude of the increases obtained was entirely out of proportion to the amount of adenylic acid injected. The increase in concentration of nucleotide expected if all of the injected nucleotide were simultaneously to appear in the blood would not exceed 0.1 to 0.2 mgm.%. The effect, therefore, appears to be an indirect one of some kind. This is further indicated by the fact that when a 100 mgm. dose of muscle adenylic acid was injected (see Table IV), the increase obtained was no greater than that obtained with the 20 mgm. dose. With the 100 mgm. dose there was a transitory increase in total purine which was not ob-

TABLE IV

Effect of intramuscular injection of 5-adenylic acid: 100 mgm. (My-B-Den) on blood nucleotides

Time (*minutes*)	AMP	ADP	ATP	Total
HA 3/1/50 Initial total purine 10.1 mgm.—30 minute total purine 11.2 mgm. %				
→0	0.0	0.9	5.7	6.6
→30	0.2	0.0	9.0	9.2
60	0.3	0.0	7.2	7.5
90	0.0	0.9	5.9	6.8
MB 2/31/50 Initial total purine 10.2 mgm. %—30 minute total purine 12.0 mgm. %				
→0	0.5	0.0	6.1	6.6
→30	0.0	0.0	9.6	9.6
60	0.3	0.5	8.3	9.1
90	0.3	4.6	4.3	9.2

TABLE V

Effect of intramuscular injection of 3-adenylic acid (yeast): 20 mgm. on blood nucleotides

Time (*minutes*)	AMP	ADP	ATP	Total
RS Total purine—12.1 mgm. %				
→0	0	0	9.0	9.0
→30	0	0	9.0	9.0
60	0	0	9.3	9.3
90	0	0	9.3	9.3
LIC Total purine—8.2 mgm. %				
→0	0	0	4.2	4.2
→30	0	0	4.5	4.5
60	0	0	4.8	4.8
90	0	0	4.8	4.8

TABLE VI

Blood cell levels of adenine nucleotides after addition of 5-adenylic acid (10 mgm. %) to whole blood in vitro

Time (*minutes*)		AMP	ADP	ATP (*mgm. %*)
0	Control	0.0	0.0	5.9
	Experimental	0.0	0.0	5.8
1	Control	0.0	0.0	5.5
	Experimental 10 mgm. % AMP	0.0	0.0	5.3
3	Control	0.0	0.0	5.2
	Experimental	0.0	0.0	5.3
15	Control	0.0	0.0	5.8
	Experimental	0.0	0.0	5.8

TABLE VII

Effect of intramuscular injection of ATP (equivalent to 20 mgm. AMP)

Time (*minutes*)	AMP	ADP	ATP	Total (*mgm. %*)
AH Total purine—10.1 mgm. %				
→0	0.0	0.0	5.3	5.3
→30	0.0	0.0	7.0	7.0
60	0.0	0.0	7.9	7.9
90	0.0	0.0	7.2	7.2

TABLE VIII

In vitro *destruction of ATP by whole blood—ATP added, 5.2 mgm. %*

Time (*minutes*)	AMP	ADP	ATP
0	0.7	0.0	4.5
15	1.3	0.6	3.3
30	1.3	0.9	3.0
60	1.8	0.6	2.8
90	3.0	0.0	2.2

served with the 20 mgm. dose. Within 60 minutes the level had returned to normal.

The possibility that the observed effects of muscle adenylic acid on blood nucleotide levels was due to psychogenic or other adventitious factors, was excluded by the results of experiments carried out under identical conditions, but substituting yeast adenylic acid for muscle adenylic acid (Table V).

That the effect of the injected adenylic acid was an indirect one was demonstrated by adding AMP directly to whole blood. The experiment was carried out in the following way. Blood was removed under heparin from the antecubital vein of one of the subjects. Ten ml. of this blood were placed in each of two tubes. One ml. from each tube was removed and centrifuged; the plasma was poured off and the cells deproteinized as before. The filtrates were assayed for AMP, ADP and ATP. To one of the blood samples was then added sufficient AMP to produce an increase of nucleotide of 10.0 mgm.%. At the end of one, three and 15 minutes, 1 ml. samples were removed, centrifuged and deproteinized as before and once more AMP, ADP and ATP were determined.

The results are shown in Table VI. It is apparent that not only does added AMP produce no effect on the distribution of other nucleotides in the cell, but it does not even appear to penetrate the red blood cell. Experiments carried out over a longer time interval gave similar results.

The injection of ATP in a dose containing the same adenine content as a My-B-Den ampule yields results which are similar to that obtained with the latter compound (Table VII). That the effect in this case may be due to the adenylic acid in ATP, rather than ATP itself is suggested by the observation that ATP placed in contact with blood is rapidly split (Table VIII).

DISCUSSION

In spite of the importance of the adenine nucleotides in so many critical life processes, this is the first report which presents data on the relative concentration of the different nucleotides in human blood. It is now possible to compare these values with corresponding values in different pathological states. Such experiments are already in progress.

From a medical standpoint it would be of great interest to know whether the reported clinical therapeutic effects of adenylic acid as well as earlier reports on ATP, are mediated through effects of this type. It would be especially interesting to know whether changes similar to those in the blood cells occur in other cells of the body (myocardium, central nervous system, nerves, glands, etc.), and whether, perhaps, the as yet unexplained actions of substances other than adenylic acid may operate through similar mechanisms. Comparison of the behavior of the adenine nucleotides in normal individuals with similar processes in various disease states may explain some of the puzzling phenomena in clinical medicine, and may possibly provide a mechanism for some of the "diseases of unknown etiology."

One point bears special emphasis: the lability of the equilibrium of the total nucleotide. We have shown that under certain conditions we can shift the balance in favor of ATP. Very recently Leder, Perlzweig, and Handler (20) have shown that when nicotinamide is added to washed erythrocytes, an eight to ten fold increase occurs in a pyridine nucleotide which behaves like DPN in a fluorometric assay. Since DPN contains adenylic acid, the latter must be derived from the same labile reservoir which under our conditions is converted to ATP. This indicates that the nucleotide may be "shifted" to compounds other than ATP under appropriate conditions.

SUMMARY

1. A technique is described for measuring the total adenine nucleotide as well as the adenylic acid, adenosine diphosphate, and adenosine triphosphate content of blood. The total nucleotide remains fairly uniform over long periods of time, while the known nucleotide (AMP, ADP and

ATP) shows considerable variation from week to week.

2. In freshly drawn blood the level of known nucleotide is maintained for at least 90 minutes when the blood is drawn under heparin; when drawn under oxalate, the ATP is rapidly converted to ADP and AMP.

3. The magnitude of the ATP level of blood is increased by the intramuscular injection of My-B-Den (muscle adenylic acid) and ATP, but not by yeast adenylic acid. The effect obtained appears to be an indirect one since larger doses produce no more of an increase than do lower doses. That the effect is indirect is further borne out by the observation that nucleotide (AMP) added directly to blood does not appear to enter the cells.

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BUTANOL-EXTRACTABLE IODINE OF SERUM [1]

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It has been demonstrated chemically that the iodine bound to proteins of the serum is predominantly composed of thyroxine (1–4). The concentration of this protein-bound or serum precipitable iodine (SPI) appears to be closely correlated with the activity of the thyroid gland (5–7). It is probably the most precise clinical criterion of such activity that has yet been extensively employed. It is, therefore, generally conceded that SPI ordinarily represents circulating thyroid hormone. In studies of medically treated hyperthyroid patients, however, a number of values for SPI proved unexpectedly high in comparison with other measures of thyroid activity (5, 7). Danowski and associates (8–10) reported that SPI of normal individuals and pregnant women rose after the administration of potassium iodide. It had also been discovered that certain iodine-containing compounds commonly used for diagnostic and therapeutic purposes formed combinations with the proteins of the serum and therefore were measured with SPI (11). For these reasons it seemed desirable to examine more closely the nature of SPI and to find a more specific measure of circulating thyroid hormone.

Taurog and Chaikoff (4) utilized *N*-butanol to extract thyroxine-like compounds from serum. This solvent, originally employed by Leland and Foster (12), can be freed from diiodotyrosine and inorganic iodine by means of an alkaline reagent suggested by Blau (13). Taurog and Chaikoff (4) found that 73 to 93 per cent of the iodine of normal plasma behaved like thyroxine inasmuch as it remained in butanol extracts after these had been washed with Blau's reagent. Danowski and associates (8–10) compared the iodine extracted by butanol with SPI in the sera of normal individuals, pregnant women, and persons taking potassium iodide. The mean value for iodine extraced by butanol in the sera from 23 healthy males and nonpregnant females and 21 pregnant women amounted to 69 per cent of SPI (10). In five normal persons the ratio, butanol-extractable iodine (BEI) : SPI varied from 0.65 to 0.82 (8). BEI remained unchanged after the administration of potassium iodide, but both SPI and inorganic iodine increased (8, 9).

By a simplification of the procedure of Taurog and Chaikoff (4), BEI of a series of subjects of varied categories has been measured and compared with SPI.

METHOD FOR DETERMINATION OF BUTANOL-EXTRACTABLE IODINE

Apparatus

Pyrex centrifuge tubes with heavy glass walls containing 25 cc. below a ground glass stopper. Holes in both the stopper and tube serve as an outlet for compressed butanol vapor.

Apparatus for digestion, distillation and colorimeter recordings of micro SPI procedure (6).

Reagents

Sulfuric acid 10 per cent by volume.

N-*Butanol,* analytical reagent.

Sodium hydroxide 4 *N.*, to each liter of which are added 50 gm. of anhydrous sodium carbonate (4, 13).

Reagents for digestion, distillation and colorimetry of micro SPI procedure (6).

Procedure

To 1 cc. of serum, mixed with 0.2 cc. of 10 per cent H_2SO_4 in the special centrifuge tubes, are added rapidly, in a fine stream, 5 cc. of butanol during continuous shaking. The mixture is shaken without the stopper to prevent loss around the ground glass joint. The tube is centrifuged at 2,000 r.p.m. for exactly eight minutes to insure a firmly packed precipitate which can be completely dispersed in subsequent portions of butanol.

After centrifugation the butanol is siphoned into a second centrifuge tube through a glass tube of such short length and small diameter as to prevent loss of butanol. To the precipitate in the original tube are added 4 cc. of butanol. Before tightening the stopper, excess vapor in the tube escapes through the vent. After vigorous shaking the stopper is removed cautiously to prevent loss of butanol. When the mixture has been centrifuged for five

[1] This investigation was supported (in part) by a research grant from the U. S. Public Health Service.

minutes the second portion of butanol is siphoned into the tube containing the original butanol extract. A third portion of 3 cc. of butanol is added to the precipitate. Shaking, centrifugation for 10 minutes and siphoning are repeated as for the second extract.

To the combined three portions of butanol is added an approximately equal volume of 4 *N*. sodium hydroxide containing 5 per cent sodium carbonate. After vigorous shaking the stopper is removed cautiously to prevent loss of butanol. The mixture is centrifuged at 2,000 r.p.m. for at least 12 minutes.

The lower layer of alkali is then removed by suction or siphoning through a tube of small diameter equipped with a stopcock which permits the tube to be inserted through the butanol layer for the removal of the alkali without admitting any butanol in the tube. Two additional extractions with the alkaline reagent are performed in a similar manner. Finally, a fourth portion of the reagent is added. After shaking, the layers are either centrifuged or allowed to separate overnight.

The butanol is drawn, with suction, into an iodine digestion flask (6). When all but a one-quarter inch layer of butanol has been removed, the ground glass stopper is held above the centrifuge tube and washed with 3 to 4 cc. of butanol. This final portion of butanol is then added to the flask containing the original extract. These final 3 to 4 cc. of butanol permit the removal of most of the thin butanol layer remaining after the first suction without admission of any of the alkali and also wash the original butanol extract from the inside of the siphon tube.

The butanol is then evaporated to dryness in a boiling water bath with suction applied through wide-glass tubes inserted in the necks of the flasks. The dried material is freed from the inside of the flask by adding 5 to 8 cc. of double distilled water. Eight cc. of 2 *N*. potassium permanganate and 18 cc. of 27 *N*. sulfuric acid are added and determination of iodine is conducted as in the steps for digestion, distillation and measurement of SPI (6).

The reagent blank is determined by extracting a mixture of 12 cc. of butanol and 0.2 cc. of 10 per cent sulfuric acid with the alkaline reagent in the same manner used in treating the butanol extract of serum. The butanol is extracted four times. After evaporation of the butanol, the visible residue of alkali is loosened by adding 5 to 8 cc. of water before the addition of a definite quantity of potassium iodide or iodate. The necessity of adding a known quantity of potassium iodide for the determination of an accurate blank has been discussed (6). The blank with the reagents employed has varied from 0.4 to 1.3 gamma per cent of iodine. It appears to arise from both the butanol and the alkali.

Notes

Considerable difficulty was encountered in extracting serum with butanol until a pH of 3.5 to 4.0 was obtained by adding sulfuric acid, using liquid brom phenol blue as an indicator. This pH is the same as that used by Taurog and Chaikoff (14, 15) and Rall (3) to extract thyroxine from an alkaline extract of thyroid gland. In the actual determination no brom phenol blue is used since it has been found that addition of 0.2 cc. of 10 per cent sulfuric acid gives the correct pH with 1 cc. portions of serum.

In Taurog and Chaikoff's (4) method the 45 cc. of butanol extracts of 3 cc. of plasma were washed with 50 cc. of alkaline reagent in separatory funnels. Decrease of the volume of 1 cc. of serum permits centrifugation and reduces the time required to layer the solutions. Preliminary separation of the organic from the inorganic iodine in serum by washing the precipitated proteins and using the washed precipitate is not necessary in this method. Values obtained by extracting untreated serum were the same as those obtained by extracting the washed zinc hydroxide precipitate of the same serum. For example, BEI's obtained from untreated serum were 7.2 and 7.3 gamma per cent, whereas BEI's obtained from the washed protein precipitate of the same serum were 7.8 and 6.9 gamma per cent (average 7.3 gamma per cent).

RESULTS

Recovery of thyroxine. Thyroxine, diiodotyrosine and inorganic iodide were added to butanol or to sera. The quantities of thyroxine added were of the magnitude that might be expected in sera from normal subjects or patients. The mixtures were then analyzed for BEI by the method described above. The sera were also analyzed by the same method before the additions. The results of these experiments appear in Table I. Multiple measurements were made to ascertain the extent of analytical variation.

Of thyroxine added to butanol in aqueous solution (Experiments 1 to 4) 93 to 102 per cent was recovered. When thyroxine was added to sera (Experiments 5 to 8), the increment recovered amounted to 88 to 103 per cent of the thyroxine added. The addition of large amounts of diiodotyrosine (Experiments 4 and 10) or a massive quantity of inorganic iodine (Experiment 9) did not affect these recoveries. Both diiodotyrosine and inorganic iodine appeared to be quantitatively removed by the washing with alkali. It had been shown earlier (16) that a fraction of diiodotyrosine adheres to protein, which is not removed by the method of washing employed in the measurement of SPI.

Although recoveries have been expressed in terms of per cent, there is nothing in these experiments to indicate that any precise fraction of thyroxine is recovered or lost. The differences between theoretical and analytical values in no instance exceed analytical errors; nor are they pro-

TABLE I

*Recovery of thyroxine iodine by butanol extraction**

Calculated iodine†			No. determinations	Determined BEI			Iodine recovered
Serum‡	Thyroxine	Total		Min.	Max.	Aver.	
γ %	γ %	γ %		γ %	γ %	γ %	%
0	5.3	5.3	3	5.0	5.1	5.1	96
0	8.5	8.5	7	7.3	8.3	7.9	93
0	10.6	10.6	2	10.2	10.6	10.4	98
0	10.4	10.4§	2	10.5	10.7	10.6	102
3.5	7.6	11.1	2	9.4	10.2	9.8	88
4.6	4.8	9.4	3	8.2	9.0	8.6	91
4.0	7.5	11.5	3	10.5	10.5	10.5	91
3.8	8.0	11.8	3	11.5	12.5	12.2	103
4.2	0	4.2‖	3	3.8	3.9	3.9	93
3.8	0	3.8§	3	2.9	4.0	3.6	95

* The thyroxine was given by Squibb Co. for investigational purposes. The iodine content by Squibb's analytical report was 64.4 per cent.
† 1 cc. samples were used for analyses.
‡ Calculated from BEI of original serum.
§ 32.0 gamma per cent diiodotyrosine iodine added.
‖ 22.4 gamma per cent diiodotyrosine iodine and 1,000 gamma per cent inorganic iodine added to serum with an SPI of 4.2 gamma per cent. Since the patient was receiving no iodine nor thio drug, the original serum was not subjected to butanol extraction.

portional to the amounts of iodine in the samples. It would be improper to conclude from these experiments that recovery was not complete. It may be that a small quantity, of the order of 0.4 gamma per cent, is lost in the course of the procedure.

BEI and SPI of normal serum. Duplicate or triplicate measurements of BEI and SPI in the sera of six normal individuals are presented in Table II. The difference, SPI − BEI, varied from 0 to 1.3 gamma per cent, averaging 0.6 gamma per cent. These differences again are of doubtful significance and not correlated with the amount of iodine present. The consistency with which SPI exceeds BEI in these and other experiments suggests, however, that the extractable iodine retained by butanol is actually slightly smaller than the SPI.

BEI and SPI of untreated patients. In Table III BEI and SPI of 17 patients with a variety of conditions are compared. None of the subjects had been given thyroid or antithyroid preparations, with the exception of Du, who had had no treatment for six months. There is reasonable doubt about B9489, who was extremely unreliable and given to self-medication. The first four were normal pregnant women. Cases 5 to 8 inclusive had large goiters. With the possible exception of B9489 all seemed to be in a euthyroid state. Cases 9 to 12 were suffering from obvious hyperthyroid-

TABLE II

SPI and BEI in normal individuals

Subject	Sex	SPI	BEI	SPI−BEI
		γ %	γ %	γ %
H	F	6.2	4.9	1.3
C	F	4.1	4.1	0
F	F	5.3	4.0	1.3
A	M	6.7	6.4	0.3
E	M	5.0	4.8	0.2
C	M	4.5	4.0	0.5
Average		5.3	4.7	0.6

TABLE III

SPI and BEI of untreated patients

Subject	SPI	BEI	SPI−BEI
	γ %	γ %	γ %
C45201	7.3	5.7	1.6
B53121	7.3	7.6	−0.3
	8.4	7.0	1.4
Du	8.4	8.2	0.2
Wa	7.7	5.7	2.0
C43177	5.0	4.0	1.0
C7178	6.9	5.8	1.1
C41885	7.0	6.0	1.0
B9489	13.7	7.8	5.9
Na	10.5	9.2	1.3
B86525	10.6	10.9	−0.3
A81700	11.5	10.8	0.7
B7958	16.6	15.1	1.5
Be	4.1	4.3	−0.2
Ma	5.8	4.7	1.1
C44978	4.6	5.1	−0.5
C32352	4.1	4.1	0
C44776	6.6	7.2	−0.6
Average			0.6

TABLE IV

SPI and BEI of hypothyroid patients taking thyroid

Subject	Thyroid	Lugol's solution	SPI	BEI	SPI−BEI
	gm./day	*gtts./day*	γ %	γ %	γ %
A79490	0.06		3.3	2.4	0.9
C9886	0.13		6.0	5.4	0.6
48533	0.13		4.3	3.9	0.4
B7257	0.13		4.5	5.0	−0.5
La	0.13		3.6	4.1	−0.5
B9007	0.13		4.2	3.2	1.0
18730	0.13		6.9	6.6	0.3
C45900	0.40		6.0	5.4	0.6
	0		3.3	3.2	0.1
	0.20		3.0	3.6	−0.6
B44180	0.33		11.0	8.9	2.1
C40160	0.06	15	9.9	6.2	3.7
A7885	0.17	14	10.8	5.1	5.7
	0.17	14	13.4	6.6	6.8

ism. The last group of five contains, in order, two young males with obesity and a woman who were suspected of hypothyroidism, a woman five or six months after thyroidectomy, and an elderly male with cerebral arteriosclerosis. The analyses in every case were made to exclude hypothyroidism. There was not satisfactory evidence in any one of them to establish this diagnosis. With one exception, B9489, mentioned above, BEI and SPI differed by no more than 2.0 gamma per cent. Again the differences are not correlated with the concentrations of iodine. BEI is usually lower than SPI, the average value of SPI − BEI being 0.6 gamma per cent.

BEI and SPI in hypothyroidism. From Table IV it can be seen that BEI and SPI of patients with hypothyroidism receiving thyroid, with one exception, differed by no more than 1.0 gamma per cent; while in three patients who were also receiving inorganic iodine the differences were 3.7, 5.7 and 6.8 gamma per cent. SPI always exceeded BEI.

BEI and SPI of patients with hyperthyroidism on medical treatment. In contrast to the data al-

TABLE V

SPI and BEI of patients with hyperthyroidism on medical treatment

Subject	Dosage for six weeks prior to study		SPI	BEI	SPI−BEI	Pulse	Serum cholesterol	Clinical impression
	Lugol's solution	Propylthiouracil						
	gtts./day	*mg./day*	γ %	γ %	γ %		*mg. %*	
Hyperthyroid								
A81700	14	200	15.9	14.1	1.8	76		Sweating. Skin fine. Tremor 2+
B20089	10*	300*	17.1	12.3	4.8	98		Sweating. Tremor 2+
Sh	10	0	10.0	7.8	2.2	88		Loss of weight
B37523	14	300	11.9	8.0	3.9	98	223	Sweating. Tremor 2+
B86525	14	300	10.9	6.4	4.5			Cardiac irregularity
C26245	14	300	12.5	8.2	4.3	96		Skin fine
Pe	15	150	13.5	8.0	5.5			
Na	14	200	12.0	8.9	3.1			
B74809	15	250	13.6	8.2	5.4			Tense
68952	0	150	6.5	7.5	−1.0			? Hyper.
A83086	0	100	8.8	8.2	0.6	92		? Hyper. Sl. weight loss
Hypothyroid								
C36063	14	300	12.4	2.2	10.2		584	Puffy dry skin. Hoarse voice
	14	0 (4 wks.)	11.8	2.3	9.5		419	Looked less hypothyroid
B59207	14	200	7.7	2.1	5.6			Puffy skin. Hoarse voice
	14	0 (2.5 wks.)	10.1	0.9	9.2		409	Cold dry skin
			12.6	0.9	11.7		398	Looked less hypothyroid
An	14	0 (2 wks.)	5.9	3.1	2.8	66		Sleepy. Wt. gain. Sl. increase in exophthalmos
C38870	14	200*	6.5	3.2	3.3		217	
	14	0 (2 wks.)	5.6	3.3	2.3			
B57397	0	300	2.5	2.3	0.2	72	265	
B92499	16	0	9.8	6.0	3.8	72	214	? Hypo. Hair normal. Skin sl. coarse. Enlarged goiter
De	15	0	7.4	2.1	5.3			? Hypo. Large goiter

TABLE V—*Continued*

Subject	Dosage for six weeks prior to study		SPI	BEI	SPI – BEI	Pulse	Serum cholesterol	Clinical impression
	Lugol's solution	Propylthiouracil						
	gtts./day	*mg./day*	*γ %*	*γ %*	*γ %*		*mg. %*	
Euthyroid								
C38870	14	0 (4 wks.)	6.6	3.4	3.2		249	
			9.4	4.6	4.8		297	
12966	14	150	11.4	4.3	7.1	80		Sl. weight gain
	14	150	5.7	1.5	4.2			
Bar	15	100	9.2	4.5	4.7			
A59918	10	400	12.3	7.1	5.2	96		Sweating. Tremor 2+
B37523	15	300	7.8	3.1	4.7	88	280	Feels better than ever before
31298	14	300	10.0	6.5	3.5			
	14	300	8.5	4.0	4.5			Constant weight
Bo	15	50	11.5	4.8	6.7			
B86525	14	300	7.2	5.9	1.3			
	14	0 (3 da.)	7.8	4.5	3.3			3 days after thyroidectomy
	0 (4 da.)	0 (1 wk.)	4.9	3.5	1.4			1 week after thyroidectomy
C20773	14	0	7.2	4.0	3.2			
51251	15	50	9.2	4.4	4.8			Asthmatic heart disease
A91311	10	150	10.2	3.7	6.5			
	10	150	13.7	4.7	9.0	72		Constant weight
A37787	5	150	14.8	8.6	6.2	80		Enormous goiter
	5	150	17.8	7.7	10.1	68		Sl. weight gain
B82863	14	100	7.5	3.7	3.8	72		Skin normal
Sc	7	100	10.6	3.6	7.0	80		
	7	200	10.6	6.0	4.6	78		Working without fatigue Adenoma seems smaller
Sco	8	150	6.2	3.2	3.0			
Mi	15	150	10.0	6.0	4.0		140	Lipiodol 2 yrs. previously
C40379	14	0	7.2	4.6	2.6	72		euthyroid
	0	0	7.1	7.4	−0.3	85		? Sl. hyper. Fine tremor
A44295	0	200	10.7	8.0	2.7	78		Weight constant
C1807	0	100	7.3	6.6	0.7			Hypertensive CV disease ? Pernicious anemia
C2869	0	100	5.8	4.8	1.0			
Sh	10	100	5.8	5.7	0.1			
Pregnant patients with hyperthyroidism								
A2544	15*	300*	15.1	9.5	5.6			Vomiting. Euthyroid
	15	300	11.2	6.7	4.5			Euthyroid. No sweating
			8.6	6.1	2.5			
B94697	15	25 (3 wks.) s-urea	10.5	7.7	2.8			Euthyroid
	15	25	11.1	6.8	4.3			Euthyroid
C24644	8	75	9.1	7.6	1.5	84		Euthyroid
	8	100	8.9	7.2	1.7			Euthyroid
98175	15	450	9.4	6.9	2.5			Euthyroid
	15	450	14.5	13.7	0.8			Actively hyperthyroid
	14	500 (2 wks.)	8.0	5.1	2.9			Euthyroid
	14	300	8.6	6.4	2.2			Euthyroid
	14	150 (2 wks.)	14.9	12.9	2.0			Hyperthyroid

* Taken irregularly.

ready shown are wide discrepancies between 64 simultaneous measurements of SPI and BEI on 40 patients receiving antithyroid drugs for hyperthyroidism (Table V). The difference between SPI and BEI may amount to 10 gamma per cent. In only 11 of the 64 instances was the difference as little as 2.0 gamma per cent, the maximum difference in subjects not on Lugol's solution and thio drugs. The normal range of SPI is between 3.8 and 7.8 gamma per cent (6). Often SPI is well above this when BEI lies in the euthyroid range or at lower values suggestive of hypothyroid-

ism. The euthyroid range for BEI has not really been established but has been considered to be 3.2 to 7.2 gamma per cent, 0.6 lower than the SPI range, because the average difference between the SPI and BEI of the six euthyroid subjects and of the 16 patients not on medication was 0.6 gamma per cent.

The clinical status of the patients in Table V was evaluated by a clinician when the blood was drawn. These evaluations have been used to classify the condition of these patients who were receiving medication for hyperthyroidism into the hyper-, hypo-, and euthyroid groups. In many instances only the final clinical impression has been included in the patient's history so that it is impossible to include notes on pulse, tremor, texture of skin and hair for every determination. After classification of clinical status the values for SPI and BEI were entered in the table.

All three patients who were still hyperthyroid according to pulse, tremor, etc., had SPI's and BEI's above the euthyroid range. Eight values on patients with mild hyperthyroid symptoms were above the normal range for SPI and BEI with one exception in each type of iodine determination. Eleven measurements on hypothyroid and questionable hypothyroid subjects were above or within the normal range for SPI, except for B57397, but below or at the lower limit of the range for BEI with one exception, B92499. The hypothyroid symptoms of B92499 were not classical and her serum cholesterol of 214 mg. per cent is compatible with euthyroidism. Elevation of serum cholesterol to values between 398 and 584 mg. per cent confirmed the diagnosis of the patients classified as definitely hypothyroid. Of the 30 measurements when patients appeared euthyroid, 25 BEI's were between 3.1 and 7.1 gamma per cent, but only 14 SPI's were between 3.8 and 7.8 gamma per cent. The BEI, therefore, appears to be a more accurate measure of thyroid activity than the SPI.

This conclusion is verified but is not as obvious in the values of the last group of Table V where are listed 11 values on four hyperthyroid patients who were pregnant and one value on B94697 two days postpartum. Except on two occasions of thyroid overactivity (98175) these patients appeared to be euthyroid. In euthyroid pregnancy the SPI is elevated to 6.0 to 10.0 gamma per cent (17). The SPI correlated closely with the clinical state of the patient except in the first value of 15.1 gamma per cent when A2544 seemed to be euthyroid. The BEI at the time of the 10 measurements, when patients appeared euthyroid, ranged between 5.1 and 9.5 gamma per cent. The range for BEI in euthyroid pregnancy appears reasonable because the SPI of normal pregnant women not on antithyroid medication was 6.0 to 10.0 gamma per cent and the BEI was only 0.6 gamma per cent lower than the SPI of euthyroid and untreated patients. When 98175 exhibited signs of thyroid overactivity, the BEI rose to 13.7 and 12.9 gamma per cent, concentrations indicative of hyperthyroidism even during pregnancy.

DISCUSSION

The reasons for the false high values of SPI are still being investigated.

The amount of propylthiouracil taken does not seem to be the single factor associated with the discrepancy between the SPI and BEI. For example, four of the largest discrepancies of 9.0 to 10.1 gamma per cent were observed in two patients, C36063 and B59207, who had stopped taking propylthiouracil two to four weeks earlier, and in two patients who were taking only 100 mg. of propylthiouracil daily. In contrast to these high differences are three below 2.9 of 98175 taking 450 or 500 mg. and four differences between 0.2 and 4.5 of four patients taking 300 mg. of propylthiouracil daily (B57397, C26245, 31298, B86525).

Differences between the SPI and BEI are obvious in patients taking Lugol's solution. This is especially apparent from the results on the 12 hypothyroid patients. The SPI and BEI agreed within 1.0 gamma per cent in nine determinations on eight patients taking either 1, 2, 3 or 6 grains of desiccated thyroid daily. But two patients who were taking Lugol's as well as desiccated thyroid had SPI's 3.7, 5.7 and 6.8 gamma per cent higher than their BEI. Seven of 10 patients on Lugol's but no propyl had SPI higher than BEI in amounts varying between 2.2 and 4.8 gamma per cent (Sh, B92499, An, C38870, C20773, C40379, B86525, C36063, B59207, De).

As yet we have only six values on patients receiving propylthiouracil but no Lugol's solution. The SPI's were 0.2, 0.6, 0.7, 1.0 and 2.7 higher and 1.0 gamma per cent lower than the BEI (A44295, B57397, 68952, A83086, C2869, C1807).

The dosage of Lugol's solution does not seem to be the factor which affects the increment between the SPI and BEI. Two patients on only 5 drops of Lugol's daily had differences between the SPI and BEI of 3.8 and 6.2 gamma per cent, while two patients on the large amount of 30 drops daily had differences of 3.8 and 7.4 gamma per cent. In 40 comparisons of SPI and BEI of patients taking 14 or 16 drops of Lugol's daily, the differences ranged from 0.8 to 10.2 gamma per cent.

The difference between the values for SPI and BEI is not the result of deterioration of serum since the BEI of two hyperthyroid patients on Lugol's and propylthiouracil was the same immediately after the blood was drawn, two, and four or five days later. In one serum the SPI was 15.9, the BEI 14.1, 14.0 and 13.5 gamma per cent. In the other blood the SPI was 11.2 and the BEI 6.7, 6.2 and 6.9 gamma per cent immediately, two and five days after the blood was drawn.

The differences between the SPI and BEI of patients on Lugol's solution can not be ascribed merely to inclusion of inorganic iodine with the precipitated serum protein. SPI has been measured before and after the addition of potassium iodide to three different sera from patients not taking inorganic iodine. Two of these fresh sera were incubated at 37° for 24 hours with 200 gamma per cent of potassium iodide. In the third experiment a similar quantity of iodide was added immediately prior to precipitation with zinc hydroxide. The concentration of 200 gamma per cent of potassium iodide had been selected because this amount or less has been observed in sera of patients on 15 to 30 drops of Lugol's daily. In each instance the SPI of the serum alone was lower than that of the serum plus iodide but the differences were 0.8, 0.8, and 1.1 gamma per cent, amounts far smaller than the differences between the SPI and BEI of the patients on Lugol's solution. For example the SPI of a patient taking 30 drops of Lugol's solution (serum inorganic iodine 225 gamma per cent) was measured in two samples of serum centrifuged four times with double distilled water and on two samples centrifuged five times. The average of those washed four times was 11.8, of those washed five times 11.3 gamma per cent in spite of a BEI of 4.4 gamma per cent. No inorganic iodine could be measured in the fifth washing and only 0.3 gamma per cent in the fourth. Danowski's observations (8, 9) are in agreement with our conclusion that the presence of inorganic iodine in serum would not in itself cause the elevation of SPI above the BEI of patients taking inorganic iodine.

It seems rather that when inorganic iodine is taken, some iodine-containing compounds in addition to thyroxine are included in the SPI. This hypothesis depends on the observation that the metabolic status of patients correlated more closely with the value for butanol-extractable iodine than with that of the SPI. Our data give no evidence of the exact chemical composition of the noncalorigenic iodine compounds included with the SPI. Diiodotyrosine is a suspect since it has been demonstrated that this compound is not entirely removed from serum proteins in the procedure for the determination of SPI (16). Monoiodotyrosine and other organic compounds containing iodine may have similar affinities for the proteins (15, 18–27).

Obviously SPI may give falsely high values for circulating thyroid hormone in patients who are receiving inorganic iodine. The conclusion has been drawn that a SPI as high as 8.0 gamma per cent is compatible with euthyroidism, although the average value for normal subjects is 5.4 gamma per cent. Engstrom and coworkers (7), in a critical analysis of the SPI of 139 hyperthyroid patients under medical treatment, found that SPI agreed with clinical criteria in 119, or 86 per cent. Discrepancies were encountered most frequently when patients became slightly or frankly hypothyroid (5). In these cases SPI did not fall below 4 gamma per cent.

Investigations are continuing to ascertain whether, as seems probable, BEI is a more accurate measure than SPI of circulating thyroid hormone.

The artifactual high values of SPI produced by priodax are also found in BEI.

CONCLUSIONS

SPI and butanol-extractable iodine (BEI) differ by no more than 2.0 gamma per cent in normal persons, pregnant women, and persons with thyroid disorders who are untreated or receiving thyroid. SPI usually exceeds BEI by an average of 0.6 gamma per cent.

In patients who have received inorganic iodine, SPI frequently exceeds BEI. The BEI seems to be more consistent than SPI with other criteria of the clinical status of such patients.

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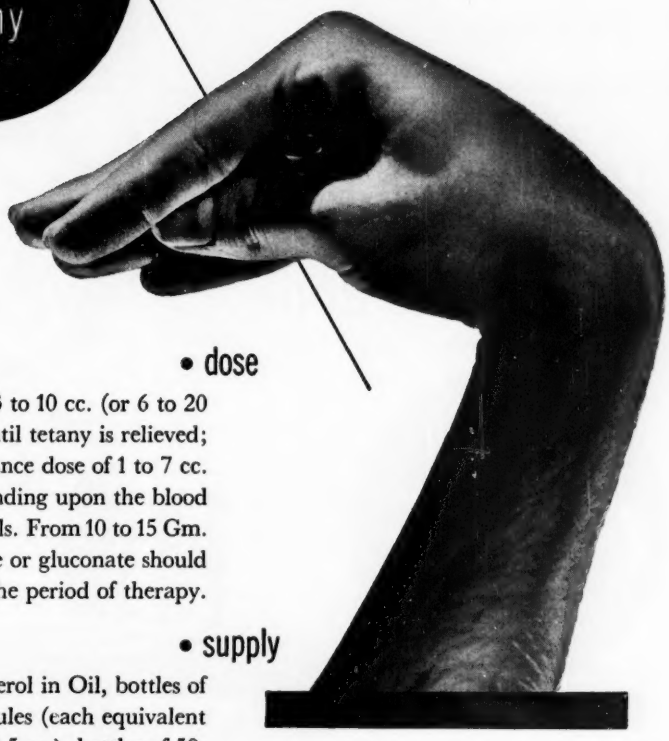

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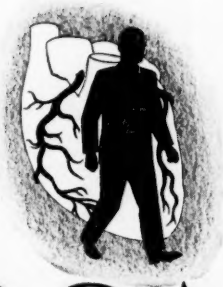

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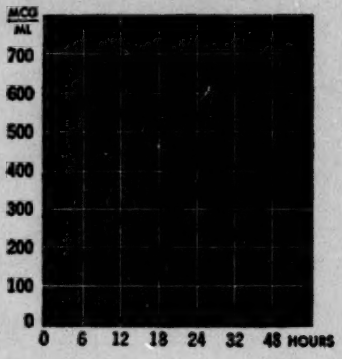

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